April 2007
Volume 6

CENTER FOR CANCER RESEARCH
frontiers
IN SCIENCE

ANNOUNCEMENT

ANNOUNCEMENT: New CCR Publication

Frontiers in Science as a stand-alone publication will be coming to an end in June 2007. CCR's intramural scientific news currently covered in *Frontiers* will be integrated into a new publication called *CCR Connections,* which will broaden communications to include external audiences as well. The new publication will highlight CCR's connectivity, its scientific interactions within and outside NCI's Intramural Research Program.

FROM THE DIRECTOR'S OFFICE

The CCR Advisory Board

The CCR Advisory Board (CAB) was established in 2005 as a mechanism through which the CCR Director, Robert Wiltrout, PhD, the Scientific Director for Clinical Research, Lee Helman, MD, and senior staff could consult CCR scientists on a variety of topics. Approximately 15 intramural scientists, including tenured, tenure-track, clinical, and basic investigators, are appointed by Drs. Wiltrout and Helman to serve on the Board for 2- to 3-year terms. The CAB meets monthly to discuss issues, give advice, and act on initiatives proposed by CCR leadership and Board members. Larry Samelson, MD, served as the first Chairperson of the CAB. He was followed by Susan Gottesman, PhD. Ron Gress, MD, will assume the role in May 2007. This article reviews several of the Board's past and present areas of focus.

Career Progress within the CCR

The first action of the Board was to update site visit guidelines, clarify the site visit process, and better inform reviewers about how the NIH differs from a university and how the site visit process differs from the grant application process. Based on recommendations by CCR leadership, changes were made in such areas as the definition of success within the Intramural Research Program, including how to evaluate participation in multidisciplinary research teams. The guidelines are now in use for CCR site visits and can be found at http://ccrintra.cancer.gov/research/site_visits.

Another concern identified through CAB discussions was how to prepare for scientific evaluations/reviews and successfully advance through the tenure-track process. Some of the

challenges raised included receipt of the appropriate mentoring and awareness of NCI/CCR resources designed to help investigators with their studies. Based on CAB discussions, coupled with recommendations from tenure-track scientists, CCR has developed new guidelines and processes for tenure-track investigators**,** including (1) holding an annual tenure-track faculty retreat, with the next scheduled for May 11, 2007; (2) assignment of a CCR Office of the Director (OD) contact for each tenure-track scientist to help with overall orientation, development of timelines for site visits, Board of Scientific Counselors (BSC) reviews, and other activities; and (3) establishment of a 2-year review of all tenure-track investigators to ensure identification of additional mentors who could provide advice on process as well as science.

Keeping CCR Science Vibrant and Scientist Morale High

To remain at the leading edge of scientific advancement, the Board has made several recommendations for areas to which future tenure-track investigators should be recruited. In addition, the CAB has developed a one-page information sheet that outlines the benefits of working in the intramural program, including logistics and funding information for potential recruits. This important information can be included in mailings that advertise positions or used for more general purposes. The Board also suggested including current CCR and NIH scientists during interviews and recruitment visits to provide a better view of the range of scientific interactions possible at the NIH, beyond the members of the search committee.

The CAB was asked for its advice in maintaining and improving morale among CCR scientists as the NCI struggles with flat budgets, new ethics guidelines, and conflict-of-interest rules. Morale is improved significantly when investigators have access to information that will directly affect their careers and research programs. Although lab chiefs meet on a regular basis with CCR leadership, direct communication between the leadership and investigators is less frequent. The CAB strongly encouraged CCR leadership to establish a forum for interaction with investigators. This suggestion is being implemented in the form of lunch meetings with Drs. Wiltrout and Helman and the CCR deputies. Small groups of investigators from different labs will meet an average of once per year for informal discussions. The Board hopes that this will provide investigators an opportunity to meet other scientists within the CCR and establish closer ties with the CCR leadership.

The CAB welcomes input from the CCR community. Please feel free to seek out or send an email to a CAB member to find out more about what the Board does and/or to suggest topics for the group's consideration. A list of current members of the Board can be found at http://ccrintra.cancer.gov/CAB.

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CARCINOGENESIS

The Role of Inflammatory Genes and NSAIDs in Colorectal Adenoma Recurrence

Sansbury LB, Bergen AW, Wanke KL, Yu B, Caporaso NE, Chatterjee N, Ratnasinghe L, Schatzkin A, Lehman TA, Kalidindi A, Modali R, and Lanza E. Inflammatory cytokine gene polymorphisms, nonsteroidal anti-inflammatory drug use, and risk of adenoma polyp recurrence in the Polyp Prevention Trial. *Cancer Epidemiol Biomarkers Prev* 15: 494–501, 2006.

The colorectal polyp is considered the main precursor lesion of colorectal cancer, and its removal during colonoscopy is thought to reduce colorectal cancer–related mortality. Approximately 30% to 40% of adults aged 60 years and older have colorectal polyps, and individuals with a history of a polyp are at increased risk of colorectal cancer. Identifying modifiable risk factors that affect the development and recurrence of these precancerous lesions is vital for colorectal cancer prevention strategies.

Chronic inflammation is a risk factor for many cancers, including colorectal cancer. The inflammatory response to cellular stresses, injury, and infection results from increased mucosal production of pro-inflammatory cytokines, which induce expression of cyclooxygenase-2 (COX-2), one of the key enzymes in the prostaglandin production. COX-2 is also involved in inflammation early in the carcinogenic pathway of colorectal cancer. However, the reported reduction in risk of colorectal polyps and cancer by nonsteroidal anti-inflammatory drug (NSAID) use never exceeds 50%, suggesting that non-responders to NSAIDs may attenuate their effect in colorectal cancer prevention. Thus, it is possible that individual genetic variations in inflammatory genes modify response to inflammation or to the chemopreventive effect of NSAIDs.

We therefore investigated the association between three single nucleotide polymorphisms (SNPs) in three different pro-inflammatory genes: *IL-1B* (–511 C/T, rs16944), *IL-6* (–174 G/C, rs1800795), and *IL-8* (–251 T/A, rs4073), two SNPs in the anti-inflammatory gene *IL-10* (–819 C/T, rs1800871 and –1082 G/A, rs1800896), and risk of adenoma recurrence. In addition, we investigated interactions between the inflammatory cytokine polymorphisms, NSAID use, and polyp recurrence.

Participants in this study were from the Polyp Prevention Trial (PPT), a multicenter randomized clinical trial to evaluate the effects of a high-fiber, high fruit and vegetable, low-fat diet on the recurrence of colorectal polyps. Briefly, men and women aged 35 years and older with a history of at least one histologically confirmed polyp removed were randomized to the dietary intervention group or the control group for 4 years. A total of 1,905 (91.6%) participants completed the study and received a colonoscopy at the fourth year. Many of them (n = 1,723, 90.4%) had DNA available for genotyping, which was performed by BioServe Biotechnologies, Ltd., Laurel, MD, via a two-step PCR process and mass spectrometry. Unconditional logistic regression was used to determine odds ratios (ORs) and 95% confidence intervals (CIs) for the association between genotype and risk of any adenoma recurrence after the 4 years of the trial, as well as risk of multiple adenoma

recurrence, adjusting for age, race, sex, and body mass index (BMI).

Overall, no statistically significant associations were found between any of the cytokine SNPs investigated in this study and risk of polyp recurrence. However, regular NSAID use for at least 3 years was inversely associated with risk of adenoma recurrence (OR = 0.70; 95% CI: 0.55, 0.90) and multiple polyp recurrence (OR = 0.55; 95% CI: 0.38, 0.80). Therefore, we examined the association of the cytokine polymorphisms and risk of polyp recurrence separately among NSAID and non-NSAID users. We observed a borderline significant increased risk of polyp recurrence among carriers of the *IL-10* –1082 A allele who were also NSAID users (OR = 1.55; 95% CI: 1.00, 2.43), as well as the suggestion of a 40% increased risk of multiple polyp recurrence. In contrast, we observed a statistically significant decreased risk of multiple polyp recurrence among non-NSAID users who were also carriers of the *IL-10* –1082 A allele (OR = 0.43; 95% CI: 0.24, 0.77) and a similar, but nonstatistically significant, 30% decreased risk of any polyp recurrence. There appears to be some antagonism between the *IL-10* –1082 G/A polymorphism and NSAID use in that the inverse odds ratios for NSAID use diminished among carriers of the *IL-10* –1082 A allele.

Our data mimic *IL-10*–deficient mice that develop spontaneous chronic inflammatory bowel disease, a known risk factor for colorectal cancer. *IL-10*–deficient (*IL-10*$^{-/-}$) mice have increased production of pro-inflammatory cytokines and several studies report that *IL-10*$^{-/-}$ mice treated with NSAIDs develop progressive, severe colitis much faster than *IL-10*$^{-/-}$ mice not treated with NSAIDs. On the other hand, NSAID-treated wild-type mice did not develop colitis and their colonic epithelium had no evidence of hyperplasia or ulcerations. Microscopic examination of NSAID-treated *IL-10*$^{-/-}$ mice revealed severe inflammatory infiltrates in their colonic mucosa and increased mRNA expression of inflammatory cytokines and COX-2 expression compared with NSAID-treated wild-type mice. It appears that inhibition of prostaglandin production was central to the development of NSAID-induced colitis. These results may help to explain our findings that individuals who used NSAIDs and were carriers of the *IL-10* –1082 A allele had a significantly increased risk of polyp recurrence. The *IL-10* –1082 A allele is associated with decreased production of the IL-10 anti-inflammatory cytokine and possibly, subsequently, an increased production of pro-inflammatory cytokines. These individuals might have enhanced production of cytokines if they also use NSAIDs and, in turn, could be at increased risk for adenoma recurrence.

Our results nominate the *IL-10* –1082 A allele as a genotype identifying individuals who may not benefit from the chemoprevention of colorectal cancer by NSAIDs. Future studies investigating the role of variants of inflammatory genes that modify the chemoprotective effect of NSAIDs may help elucidate the biological mechanisms of colorectal cancer and identify individuals who will respond best to these chemopreventative agents. Such studies might also aid in the development of public health and clinical intervention programs aimed at preventing colorectal cancer.

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CELL BIOLOGY

Poor Response of Malignant Melanomas to Chemotherapy Is Linked to Melanosomes

Chen KG, Valencia JC, Lai B, Zhang G, Paterson JK, Rouzaud F, Berens W, Wincovitch SM, Garfield SH, Leapman RD, Hearing VJ, and Gottesman MM. Melanosomal sequestration of cytotoxic drugs contributes to the intractability of malignant melanomas. *Proc Natl Acad Sci U S A* 103: 9903–7, 2006.

Malignant melanomas are notorious for their resistance to treatments such as radiation and chemotherapy. According to the American Cancer Society, approximately 62,000 new melanoma cases were diagnosed in the United States in 2006, and 7,900 people died of this disease, the fifth most deadly of American cancers. Until now, the precise mechanisms that underlie therapeutic resistance in melanomas remained elusive. More researchers are currently focusing on new, promising therapeutic approaches, such as immunochemotherapy, in an attempt to improve the survival rate of patients with the disease.

Clearly, determining the predominant drug resistance mechanisms is a key step in developing effective therapies. The major cellular/structural difference between melanoma and non-melanoma cancer cells lies in a cytoplasmic organelle called the melanosome. Melanosomes are unique membrane-bound compartments adapted for melanin synthesis in pigment-producing cells, including melanocytes and melanoma cells. Melanosomes also store toxic intermediates produced during melanin synthesis. In this study, we examined the role of melanosomes in drug resistance by directly comparing the melanosomal sequestration of cytotoxic drugs such as *cis*-diaminedichloroplatinum II (CDDP) in MNT-1 melanoma cells and in KB-3-1 epidermoid carcinoma cells.

We initially observed intracellular accumulation of a fluorescent dye (Alexa-Fluor)–labeled platinum compound (designated as AF-CP) in the cytoplasm, but not in the nuclei, of MNT-1 cells. In contrast, KB-3-1 cells accumulated significant amounts of AF-CP in both the cytoplasm and the nuclei. Using immunofluorescence confocal analysis, we colocalized AF-CP with a stage II melanosome marker (i.e., HMB-45) in melanosomes. In our previous study (Liang XJ et al. *J Cell Physiol* 202: 635–41, 2005), we also found that AF-CP reflects

at least some biological properties of unmodified CDDP. Thus, we reasoned that the cytoplasmic/melanosomal trapping of AF-CP in melanoma cells likely reflects some properties of chemotherapeutic drugs such as CDDP used in the treatment of melanoma.

To verify the results obtained from the experiments with AF-CP, we used an X-ray probe to directly map the intracellular retention of the platinum compound (which for study purposes we regarded as unmodified CDDP) both in MNT-1 cells and in KB-3-1 cells. We found that the nuclear retention of CDDP in MNT-1 cells was much less than that which we observed in KB-3-1 control cells. The melanosomal localization of CDDP was also confirmed by melanosomal emission spectrum analysis of platinum. Hence, we were able to colocalize more than 50% of CDDP within melanosomes. These data indicate that the platinum-containing compounds are trapped mainly in subcellular organelles such as melanosomes. Our data thus suggest a fundamental difference between melanoma and nonmelanoma cells in terms of their cytoplasmic/melanosomal and nuclear drug distributions. Clearly, this difference could explain differential chemosensitivity in *in vitro* cellular models and perhaps in melanoma patients.

We further found that melanosome biogenesis could influence the melanosomal localization of AF-CP. In particular, an increase in the generation of stage II or stage II–III melanosomes, but not stage IV melanosomes (the highly pigmented organelles), might significantly change melanosomal drug trapping. Moreover, melanosome biogenesis can be enhanced by various anticancer drugs such as CDDP and vinblastine, which possess different modes of action on their cellular targets. Cytotoxic drug treatment of melanoma cells also caused elevated pigmentation and accelerated melanosome export. Since melanocytes are biologically primed to extrude melanosomes as part of the skin pigmentation process, we speculate that extrusion of drug-containing melanosomes by melanomas also contributes to their relative drug resistance. We found no correlation between melanin content and drug resistance among melanoma cell lines, suggesting that it is the melanosomes per se and not their content of melanin that mediates resistance. We are currently exploring the role of transport systems in enhancing accumulation of cytotoxic drugs in melanosomes as the first step in this novel drug resistance process.

In summary, our studies indicate that melanosome numbers, melanosomal trapping, and melanosome export are involved in drug resistance in melanoma (Figure 1). Cytotoxic drugs such as CDDP can be trapped in subcellular organelles such as melanosomes, thus reducing the drugs' cytotoxicity. Moreover, some cytotoxic drugs can also regulate melanosome numbers as well as pigmentation, which in turn enhances melanosome-mediated drug trapping and accelerates melanosome export. Therefore, our studies provide possible therapeutic approaches to circumventing multidrug resistance in melanomas via inhibition of melanosome biogenesis and melanosome-mediated drug trapping and/or export.

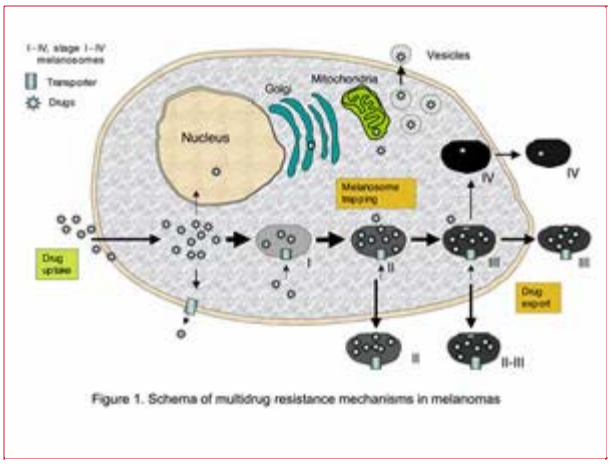

Figure 1. Schema of multidrug resistance mechanisms in melanomas.

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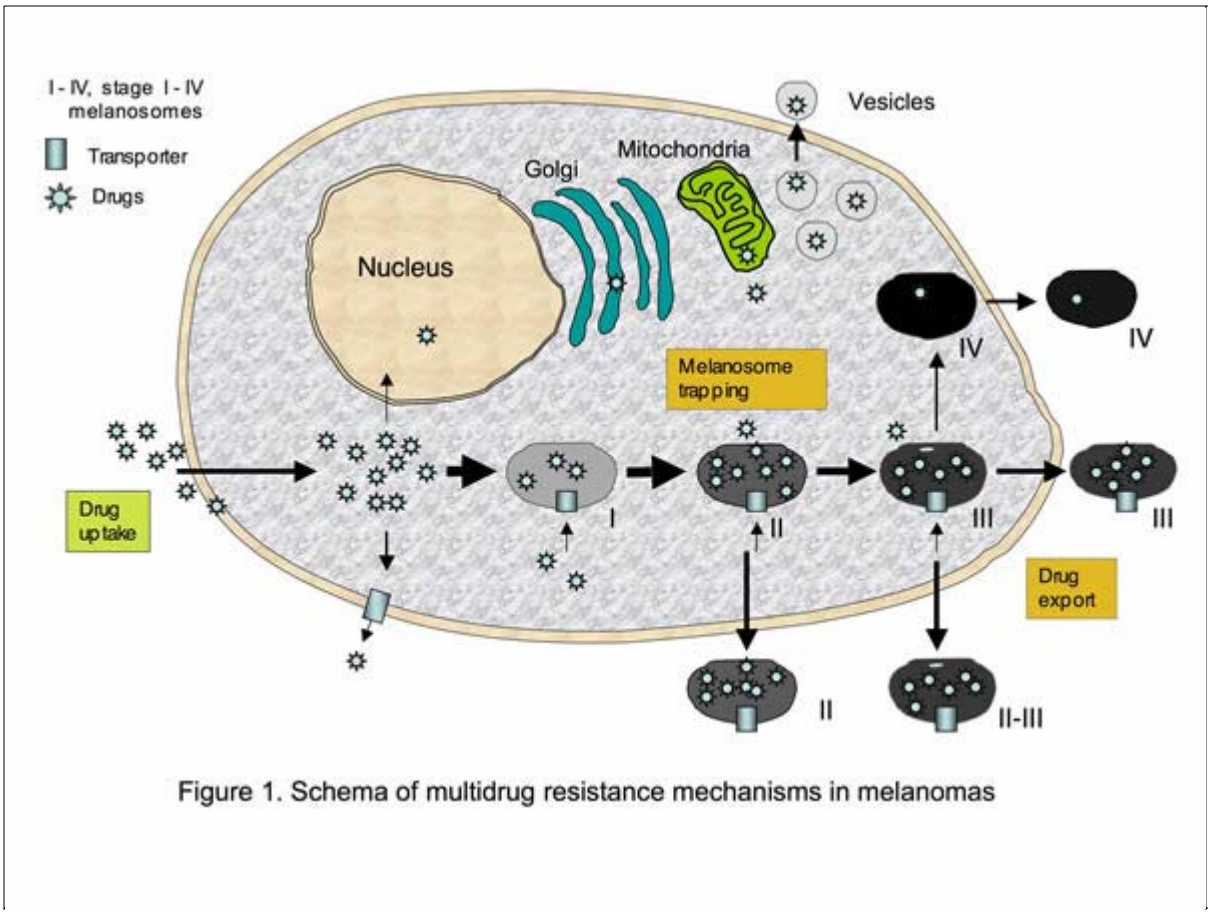

Figure 1. Schema of multidrug resistance mechanisms in melanomas

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MOLECULAR BIOLOGY

Thioredoxin Reductase 1: A Double-edged Sword in Cancer Prevention and Promotion

Yoo MH, Xu XM, Carlson BA, Gladyshev VN, and Hatfield DL. Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. *J Biol Chem* 281: 13005–8, 2006.

Dietary selenium has potent cancer prevention activity. Both low–molecular-weight selenocompounds and selenoproteins have been implicated in this activity. The major effect of selenium in health, however, is likely through the action of selenoproteins (Hatfield DL, Berry MJ, Gladyshev VN [Eds]. *Selenium: Its Molecular Biology and Role in Human Health.* Springer: New York, NY, 2006). Thioredoxin reductase 1 (TR1) is one of 25 known selenoproteins in humans and is a major antioxidant and redox regulator in

mammalian cells. Interestingly, this enzyme appears to have opposing effects in cancer development, as it has been implicated in both cancer prevention and cancer promotion. For example, TR1 supports p53 function and has other tumor suppressor activities, and its inhibition by carcinogenic, electrophilic compounds further suggests a role in cancer prevention. On the other hand, TR1 is overexpressed in many cancer cells, and its inhibition by a variety of potent agents has been shown to alter the cancer-related properties of numerous tumors and malignant cells, leading several investigators to propose this enzyme as a possible target for cancer therapy.

It is not clear whether the cancer-preventing or cancer-promoting properties of TR1 influence cancer development more. To help determine this, we directly examined the role of this enzyme in a cancer cell line and in a mouse model.

We used RNA interference to specifically target and knockdown TR1 activity. Mouse Lewis lung carcinoma (LLC1) cells were stably transfected with the target vector and a control vector that had the same DNA sequence but lacked the targeting sequence. Two separate sites within the 3′-untranslated region of *TR1* were initially targeted because they were found to have very similar effects on reducing TR1 expression, which ruled out any possibility of off-targeting. The level of TR1 was substantially reduced in both these knockdown cell lines compared with the control cell line, as determined by Northern and Western blot analyses, 75-selenium labeling that specifically labeled the selenocysteine residue in TR1 (and other selenoproteins), and by direct assay of enzyme activity.

LLC1 cells transfected with the TR1 target had a number of altered properties that were more in line with normal cells than with the LLC1 cells transfected with the control vector. For example, the TR1-deficient cells manifested a retarded growth rate compared with control cells. Other characteristics of LLC1 cells were also altered: For example, the control cells grew to be multilayered and loosely attached to the culture dishes. They also grew non-anchored in soft agar. In contrast, the TR1-inhibited cells grew in monolayers and were tightly attached to culture dishes. Also, their growth in soft agar was inhibited. Moreover, the expression of at least two cancer-related mRNAs, those of hepatocyte growth factor and osteopontin, was substantially reduced in TR1-inhibited cells.

Most significantly, mice injected with LLC1 cells that carried the TR1-targeting vector manifested a dramatic reduction in tumor progression and metastasis compared with mice injected with cells carrying the control vector. Tumorigenesis was examined by injecting three mice in the flank with TR1 knockdown cells and three mice with control cells. After two weeks, the mice were euthanized, the tumors excised and weighed, and the weights averaged. Tumors were much larger in mice injected with control cells, with an average weight of 0.341 g compared with an average weight of 0.063 g in mice injected with the TR1 knockdown cells. Moreover, the smaller tumors that arose from the TR1-deficient cells had lost the targeting vector, suggesting that TR1 is essential for tumor growth. Tumor metastasis was analyzed by injecting tail veins from mice with TR1 knockdown cells and control cells. The mice that received the injections were euthanized after 4 weeks, and their lungs were removed. Lungs from mice injected with the control cells had extensive tumor formation,

whereas the lungs from mice injected with the TR1 knockdown cells had no visible tumors. Pathological analysis of lung slices showed widespread malignancy in mice injected with control cells, but only normal tissue in mice injected with the TR1 knockdown cells.

Overall, our study demonstrated that downregulating TR1 expression reverts the phenotype of malignant cells, making it more in line with that of normal cells. These observations provide direct evidence that the reduction of TR1 levels in malignant cells is antitumorigenic.

How can this apparently essential function of TR1 in cancer development be reconciled with the role of this enzyme in tumor suppression as well as the known anti-cancer role of selenium, which is a catalytic component of TR1? We propose that an adequate amount of dietary selenium in general, and a normal expression level of TR1 in particular, maintain cellular redox homeostasis in normal cells, protecting them against oxidative stress, DNA mutations, and damage to other cellular components. Each of these roles of selenium and TR1 are functions in which both components have been implicated. However, in newly emerging tumors, TR1 would be required to sustain tumor growth, likely because of the increased demand for its reducing equivalents. All of this would explain both the potent cancer prevention activity of dietary selenium and the role of TR1 as a double-edged sword in preventing and promoting cancer. Furthermore, our study provides the basis to explain disparate data in the literature on the role of this enigmatic protein in cancer and elevates TR1 to a prime target for cancer therapy.

Such studies as the present one are having a major impact on how we envision the dietary intake of selenium in humans and other mammals. It has been known for some time that diets containing sufficient or supplemental amounts of selenium have beneficial effects in preventing certain forms of cancer, possibly through the action of enriching the selenoprotein population. However, once cancer is initiated, then adequate or enriched amounts of selenium in the diet might serve to drive tumorigenesis.

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MOLECULAR BIOLOGY

Measuring *In Vivo* Binding

Sprague BL, Muller F, Pego RL, Bungay PM, Stavreva DA, and McNally JG. Analysis of binding at a single spatially localized cluster of binding sites by fluorescence recovery after photobleaching. *Biophys J* 91: 1169–91, 2006.

For many years, an assortment of established biochemical techniques have provided *in*

vitro estimates of the binding affinity of different proteins to cellular target molecules. However, it has been impossible to determine how close these *in vitro* affinity measurements are to the actual *in vivo* affinities, simply because there have been no established methods for measuring binding affinities within live cells. Techniques for this are now becoming available due to advances in light microscopy and, interestingly, the first results reveal striking differences compared with the *in vitro* estimates.

One approach for measurement of *in vivo* binding is fluorescence recovery after photobleaching (FRAP). This technique is performed by photobleaching fluorescent molecules at a specified location in a cell, and then monitoring the rate at which the bleached molecules are replaced by unbleached ones. This recovery rate will be influenced by the rate at which the fluorescent molecule diffuses. In addition, if the fluorescent molecule binds to a relatively immobile substrate such as DNA, then the recovery rate will also be affected by the strength of that binding interaction. Tightly bound molecules will yield much slower FRAPs than weakly bound molecules.

To tease out the contribution of diffusion and binding from FRAP data, mathematical models are required that account for both processes. The equations describing a FRAP recovery include a term for diffusion, plus chemical kinetic terms for the on and off rates of binding to an immobile substrate. These equations can be used to predict FRAP recoveries, and therefore determine which combination of diffusion constant, on rate, and off rate will yield the best match to experimental FRAP data.

We have developed, analyzed, and applied such model equations to FRAP data for the glucocorticoid receptor, a transcription factor that resides in the cell nucleus after exposure to steroid hormone. In earlier work, we showed that binding of the glucocorticoid receptor to specific promoter sequences could be visualized in live cells containing an array of 200 tandemly repeated copies of the promoter and downstream reporter genes stably integrated into a mouse chromosome. A series of control experiments demonstrated that transcription occurs normally from this promoter array, which appears as a bright spot within a cell nucleus containing a green fluorescent protein (GFP)–tagged glucocorticoid receptor.

FRAP experiments performed at the promoter array will therefore yield recovery data containing information about the *in vivo* binding of the glucocorticoid receptor to the promoter sites. To estimate these *in vivo* binding parameters, we developed a mathematical model to account for FRAP at a spatially localized cluster of binding sites. The resultant partial differential equations were solved numerically using a finite element method, and then used to predict FRAP curves at the promoter array.

With this model, we obtained excellent fits of the experimental FRAP data using only a single, free parameter, namely the ratio of the on to off rates of binding at the promoter sites. This yielded an estimate for an *in vivo* binding constant of 10^{-7} M, and an upper limit of 170 milliseconds for the residence time of a glucocorticoid receptor on a promoter. In stark contrast, *in vitro* binding estimates have yielded binding constants from 10^{-8} to 10^{-10} M and residence times of 90 minutes.

These striking differences almost certainly reflect, at least in part, the fact that the *in vitro* experiments were performed with naked DNA and purified glucocorticoid receptor, whereas *in vivo,* the promoter DNA is packaged as chromatin and the glucocorticoid receptor may be associated with a variety of cofactors that could influence its binding. However, since techniques for measurement of *in vivo* binding are still in their infancy, further work is needed to establish the validity of the mathematical models.

We have begun this process by incorporating a number of real-life features into the models, and then comparing the results of these more complex models to the results of simpler ones. This has shown that some assumptions have serious consequences, whereas others have insignificant effects and so in general can be ignored.

Although further computational tests will help refine the mathematical models, independent techniques for measuring *in vivo* binding will also be necessary to validate the FRAP estimates. Fortunately, several interesting complementary approaches, such as fluorescence correlation spectroscopy, are also being developed to estimate *in vivo* binding parameters. As these various techniques are refined and are shown to yield similar estimates for live-cell binding parameters, we will have moved a step closer to a new era of *in vivo* biochemistry by light microscopy.

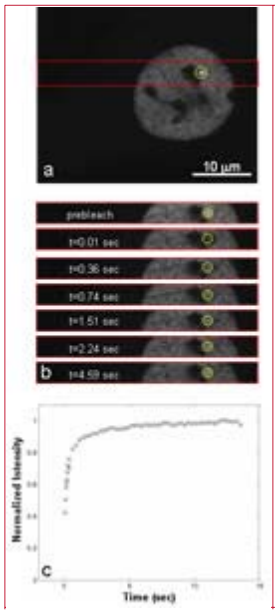

Figure 1. A live cell nucleus containing hormone-induced green fluorescent protein–tagged glucocorticoid receptors (GFP-GR) and 200 tandemly repeated copies of a promoter array for GR *(a).* The array appears as a bright spot marked here by the yellow circle. In fluorescence recovery after photobleaching (FRAP) experiments, fluorescence is specifically bleached only inside the yellow circle, and then the rate at which fluorescence recovers there is monitored *(b).* Since the rate of fluorescence recovery is rapid, narrow strip images (red rectangle in part *a* above) are acquired on a confocal microscope, thereby reducing the scan time for acquisition of each image during the fluorescence recovery. Images at a few selected recovery time points are shown *(b).* Using all the collected time points, the average intensity inside the yellow circle is measured to generate a FRAP curve *(c).* The curve is normalized to one based on the initial intensity inside the yellow circle. Curves such as this can be fit to estimate values for the *in vivo* binding parameters of GR at a promoter.

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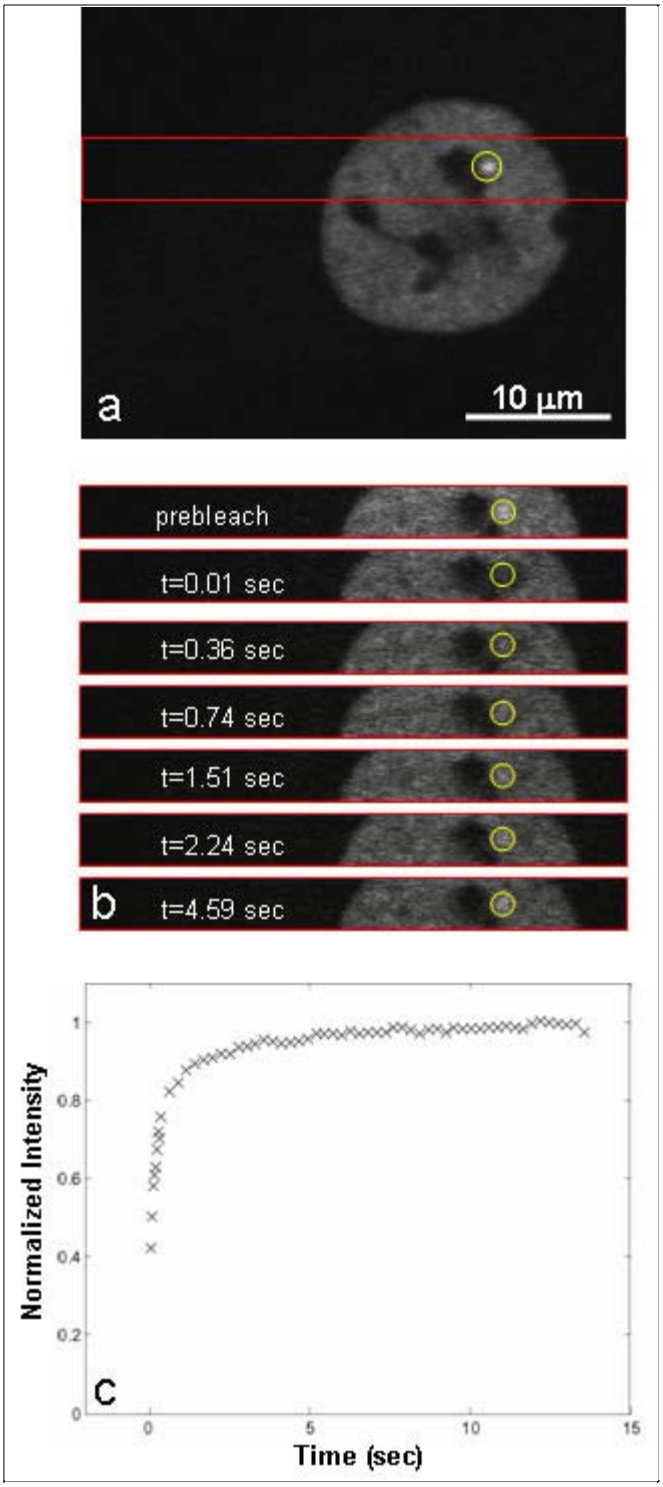

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MOLECULAR BIOLOGY

A Catalogue of Structural Motifs in Amyloid Fibril Organization

Zheng J, Ma B, Tsai CJ, and Nussinov R. Structural stability and dynamics of an amyloid-forming peptide GNNQQNY from the yeast prion sup-35. *Biophys J* 91: 824–33, 2006.

A wide variety of proteins with no sequence similarity or structural homology may form linear, unbranched fibrils sharing specific cross-β sheet structures. Many of these are associated with neurodegenerative diseases. Determination of high-resolution molecular structures of amyloid fibrils is an important first step toward understanding the pathogenesis and aggregation mechanism of amyloid diseases, yet it is still a highly challenging task due to the noncrystalline and insoluble nature of amyloid fibrils. Nevertheless, the combination of computational predictions (Ma B and Nussinov R. *Proc Natl Acad Sci U S A* 99: 14126–31, 2002; Zheng J et al. *Biophys J* 91: 824–33, 2006) and experimental results (Nelson R et al. *Nature* 435: 773–8, 2005; Luhrs T et al. *Proc Natl Acad Sci U S A* 102: 17342–7, 2005; Petkova AT et al. *Biochemistry* 45: 498–512, 2006) allows derivation of the emerging structural motifs in amyloid organization (summarized in Table 1). A catalogue of these structural motifs is expected to be enormously useful in drug design for prevention and treatment of amyloid-related diseases.

Table 1. General Structural Features in Amyloid Fibrils

Feature	Characteristics

Sheet-to-sheet recognition via steric zipper	Between β sheets, an inter-sheet zipper can be characterized by complementarity—of shape, hydrophobicity, charge, and hydrogen bonding.
Twisted cross-β sheet	The twist angle involved in single and multiple β sheets can range between approximately 5 and 20 degrees.
Identical/similar residue ladder in a parallel in-register organization	Asn and Gln ladders; aromatic stacking (Phe, Tyr, Pro, and His); hydrophobic stacking (Val, Ile, or Leu).
β-strand-loop-β-strand	Two adjacent β strands in the primary sequence oriented in an antiparallel arrangement and linked by a short loop of two to five amino acids.

Steric Zipper. There is no universal driving force that associates and stabilizes β sheets into amyloid fibrils: Hydrophobic interactions govern some cases (Aβ, human islet amyloid, and Syrian hamster prion protein), whereas polar interactions govern others (GNNQQNY and human calcitonin amyloid). Yet, by examining crystal structures of amyloid peptides, including GNNQQNY, Aβ, and human CA150, we observed a similar steric zipper arrangement in those protofilaments. The remarkable GNNQQNY crystal structure from the yeast protein Sup35 presents a dry, tightly self-complementing steric zipper between two β sheets. Simulations of mutational variants show that substitutions of N2, Q4, or N6 by Ala at the dry interface knock down the steric zippers, destroy sheet-sheet packing, and thus inhibit fibril formation. Similarly, Aβ and human CA150 amyloidogenic peptides form steric zippers by the interdigitation of side-chains (i.e., M35-M35 contacts for Aβ and T13-T18, V5-R24, V5-L26, and T3-S28 contacts for CA150). Since the shape-complementary zipper optimizes side-chain and main-chain interactions common to amyloid fibrils *regardless* of their sequences, the steric zipper may be a general feature in amyloid fibrils. The zipper can be hydrophobic or polar and the interactions within or between molecules.

Twisted Cross-β Sheets.The simulations indicated that the GNNQQNY β sheets twist by about 15 degrees. The twisted sheets are not unique to GNNQQNY; rather, similar twisted β sheets were observed in other amyloid peptides such as $A\beta_{16\text{-}22}$ (KLVFFAE), $A\beta_{21\text{-}30}$, the human islet amyloid polypeptide$_{22\text{-}27}$ (NFGAIL), KFFE, KVVE, KLLE, KAAE, the human calcitonin hormone$_{15\text{-}19}$ (DFNKF), and NHVTLSQ from human β_2-microglobulin. Since twisted β sheets optimize the hydrogen bonds, side chain stacking, and electrostatic interactions, it is commonly accepted that twisted sheets are more stable than flat sheets. Interestingly, the pairs of β sheets, while twisting, are still compatible with the steric zipper.

Parallel β Sheet Organization. Many amyloid fibrils consist of parallel β sheet structures, at least for longer protein chains or peptides. Such a parallel organization, observed for GNNQQNY, Aβ, the human CA150 WW domain, β_2-microglobulin, and other peptides, as well as in β-helices, allows a ladder-like stacking of chemically similar side chains on top of each other (e.g., Asn or Gln ladders, aromatic stacking, and hydrogen bonding zipper).

Unlike in antiparallel organization, shuffling the sequence is not likely to disrupt those residue pairs and thus has little impact on parallel β sheet structures.

β-Strand-Loop-β-Strand. The β-strand-loop-β-strand motif is formed by two β strands of non-native register linked by a flexible loop. As first predicted by the simulations of Aβ (Ma B and Nussinov R. *Proc Natl Acad Sci U S A* 99: 14126–31, 2002), the β-strand-loop-β-strand motif was recently discovered in the amyloid protofilaments of human CA150 and the β_2-microglobulin. This motif consists of two sheets whose side chains zip against each other in an antiparallel fashion, where each sheet consists of a parallel arrangement of the β strands. The loop is stabilized by a salt bridge in Aβ and covalent bonds in CA150 and β_2-microglobulin. Thus, this motif resembles those of shorter peptides, illustrating the advantage of the tight packing.

Although our current work has made progress in understanding the dynamics and structure (thermodynamics) of amyloid formation, it behooves us to remember that the details of peptide organization and preference among possible conformational states depend on amino acid composition, sequence, chain length, and environment. Further, for a given sequence, amyloids are likely to exist as different phenotypic strains; that is, there may be meta-stable conformational states obeying the same conformational principles. Moreover, beyond the motifs, the crucial questions of the kinetics and pathways of amyloid formation and the mechanism of amyloid toxicity still remain; our studies of these key issues are under way.

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