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■ MOLECULAR BIOLOGY

Coupling RNA Polymerase's Distribution to Global Gene Regulation and Chromosome Condensation

Cabrera JE and Jin DJ. The distribution of RNA polymerase in *Escherichia coli* is dynamic and sensitive to environmental cues. *Mol Microbiol* 50: 1493-505, 2003.

Escherichia coli RNA polymerase (RNAP) is a multisubunit enzyme. Unlike in eukaryotes, in which three different RNAPs (Pol I, II, and III) synthesize three different RNA species (rRNA, mRNA, and tRNA/5S rRNA, respectively), a single RNAP synthesizes all RNA species in *E. coli*, doing so in response to specific growth conditions. Under optimal growth conditions, for example, the vast majority of RNAP molecules synthesize rRNA and tRNA (termed stable RNAs). These forms of RNA are encoded by genes that represent less than 1% of the genome. The fewer, remaining RNAP molecules (that is, those not synthesizing stable RNAs) are responsible for synthesizing mRNA in the whole genome.

Under suboptimal conditions, such as when cells are growing slowly in nutrient-poor media, few RNAP molecules synthesize stable RNAs. When cells are shifted from nutrient-rich to starvation conditions, such as amino acid deprivation leading to what is termed the stringent response (Cashel M et al. In *Escherichia coli and Salmonella typhimurium* 1458-96, 1996), the cellular transcription machinery is dramatically reprogrammed in such a way that the expression of stable RNAs is totally

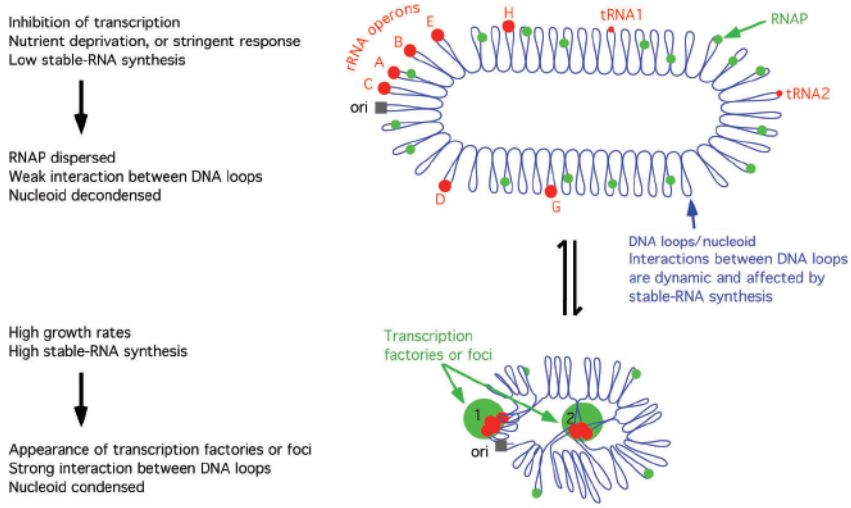

Figure 1. Model of stable-RNA synthesis, transcription factories or foci, and chromosome condensation. The *Escherichia coli* chromosome is represented as blue lanes folded in loops, the ori (origin) of replication as a black square, the seven rRNA operons as large red circles with letters, and two representative tRNA operons as small red circles. The RNA polymerase (RNAP) molecules are represented as small green circles. For simplicity, only two putative transcription factories or foci, which make the nucleoid more compact by pulling different stable RNA operons into proximity, are indicated here (bottom part of the diagram, large green circles labeled 1 and 2).

inhibited while that of amino acid biosynthetic operons is activated.

Despite extensive genetic, biochemical, and structural studies on RNAP, little is known about the location and distribution of the molecule in *E. coli* under different physiologic conditions. We constructed a functional *rpoC-gfp* gene fusion on the *E. coli* chromosome to visualize RNAP in the cell by fluorescence microscopy under different growth conditions. Visualization of RNAP proved to be challenging, however, mainly because *E. coli* cells mounted on microscope slides have a different physiologic state than those growing in a culture. To ensure accurate snapshots of the RNAP distribution/location under different growth conditions, a modified sampling procedure was successfully developed: the cells being examined were rapidly fixed with formaldehyde to "freeze" RNAP and subcellular structures before imaging.

Several conclusions have been derived from this study. First, RNAP is located exclusively either within and/or surrounding the nucleoid (i.e., there is no RNAP-GFP signal in cytoplasmic space). As the study used only a two-dimensional imaging technique, those RNAPs apparently located within the nucleoid could in fact have been surrounding the nucleoid. If this is the case, RNAP in the location would facilitate the coupling between transcription and translation (or ribosome assembly). Three-dimensional imaging techniques are warranted to resolve this issue.

Second, growth conditions, nutrient deprivation, and transcription activity affect the distribution of RNAP. In fast-growing cells cultured in rich medium, RNAP distribution was heterogeneous, being concentrated in areas of the nucleoid. These areas were named transcription foci because they disappeared in the presence of the antibiotic rifampicin, inhibiting transcription. The transcription foci are likely RNAP molecules actively engaged in stable-RNA synthesis, because in slow-growing cells cultured in nutrient-poor media, the distribution of RNAP was relatively homogeneous and transcription foci were not evident. Also, while the transcription foci disappeared rapidly in wild-type cells during amino acid starvation, they remained present in an isogenic *relA* mutant strain in which stable RNAs were still actively being synthesized (Metzger S et al. *J Biol Chem* 264: 21146-52, 1989). Moreover, the distribution of a "stringent" RNAP in an *rpoB* mutant, in which stable-RNA synthesis was impaired even in rich media (Zhou YN et al. *Proc Natl Acad Sci U S A* 95: 2908-13, 1998) resembled that of wild-type RNAP during the stringent response. Thus, we proposed that the transcription foci are transcription "factories" synthesizing stable RNAs, which form a structure(s) analogous to the eukaryotic nucleolus (Cook PR. *Science* 284: 1790-5, 1999).

Finally, the synthesis of stable RNAs is apparently a driving force in the condensation of the *E. coli* chromosome. The nucleoids became decondensed in wild-type cells when stable-RNA synthesis was preferentially inhibited during the stringent response, whereas they remained condensed in the *relA* mutant cells where stable-RNA synthesis was maintained during amino acid starvation. Moreover, the nucleoids were decondensed in the "stringent" RNAP mutant defective in stable-RNA synthesis even when grown in nutrient-rich media. From these data, a working model was proposed to link the synthesis of stable RNAs, RNAP distribution, and chromosome condensation in bacteria (Figure 1).

In summary, the distribution of *E. coli* RNAP is dynamic and sensitive to physiologic changes. This study not only reveals a link between global gene regulation, such as the stringent (nutrient deprivation) response, and RNAP's (re)distribution in cells, but also an important role played by RNAP actively engaged in the synthesis of stable RNAs, in particular rRNA, in forming transcription factories or foci and bringing about chromosome condensation.

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■ IMMUNOLOGY

Interleukin 15 in Cellular Communication

Dubois S, Mariner J, Waldmann TA, and Tagaya Y. IL-15Rα recycles and presents IL-15 *in trans* to neighboring cells. *Immunity* 17: 537-47, 2002.

Interleukin 15 (IL-15) is a factor pivotally involved in the development and homeostasis of natural killer (NK) cells and CD8 memory T cells. How IL-15 acts on these cells is unclear, even though IL-15 functions *in vivo* are fairly well understood. The current thinking is that cells need to co-express three IL-15 receptor subunits (IL-15Rα, IL-2/IL-15Rβ, and γ_c) to respond to physiological concentrations of IL-15. However, resting NK cells and naïve CD8 T cells do not express IL-15Rα. Additionally, IL-2 and IL-15, which share the signaling components (β and γ_c) of their receptors, show very distinct functions *in vivo*. IL-15Rα thus appears to have a unique role.

When IL-15Rα was overexpressed on a cytokine-dependent T-cell line (CTLL-2), we observed that the cells survived longer (48 to 72 hours) after IL-15 withdrawal from the culture than did parental cells that express minimal amounts of

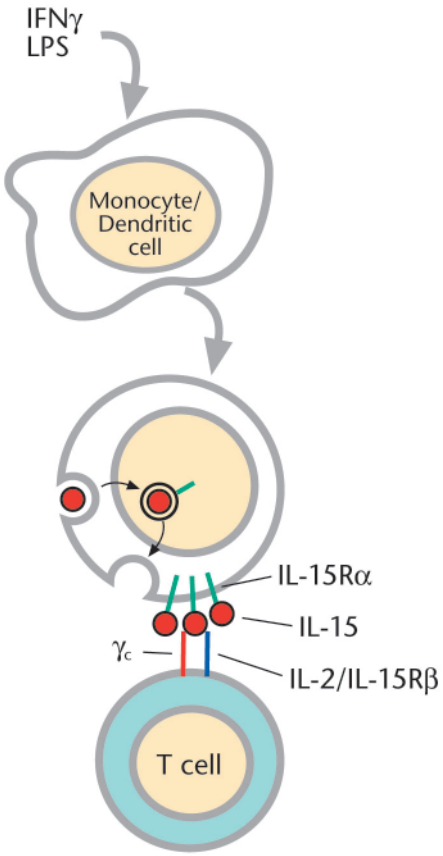

Figure 1. Interleukin 15 receptor α (IL-15Rα) on antigen-presenting cells presents interleukin 15 (IL-15) *in trans* to natural killer cells and CD8 memory T cells. *Top:* Interferon γ (IFNγ) and lipopolysaccharide (LPS) stimulate the coordinated expression of IL-15Rα and IL-15R. *Middle:* IL-15Rα and IL-15R recycle in endocytotic vesicles. *Bottom:* IL-15Rα presents IL-15R *in trans* to T cells expressing the IL-2/IL-15 receptor signaling components β and γc.

IL-15Rα. This observation suggests that the overexpression of IL-15Rα played a role in the persistence of IL-15 action. We hypothesized that IL-15Rα and IL-15 form a stable complex on the cell surface. We confirmed this hypothesis by expressing IL-15Rα on 293 cells, incubating the transfected cells with IL-15, then demonstrating the presence of IL-15 on the cell surface by flow cytometry using an anti–IL-15 antibody.

The IL-15/IL-15Rα complexes on cell surfaces have distinctive characteristics. First, the complexes undergo endosomal processing, leading to the persistence of a stable complex on the cell surface. Stripping cell surface IL-15 from the complex by exposing the cells to acidic conditions (pH 3) results in the reappearance of cell surface IL-15 after 6 to 12 hours of re-incubation in cytokine-free medium at 37°C. This observation indicates the intracellularly stored IL-15/IL-15Rα complexes are recycled back to the cell surface, providing a new source for cell surface IL-15.

Second, cell surface IL-15/IL-15Rα complexes allow cells to present biologically active IL-15 *in trans* to neighboring cells. In particular, physiological concentrations of IL-15 are captured by IL-15Rα and then presented *in trans* to activate target cells that express IL-2/IL-15Rβ but not IL-15Rα (Figure 1). Because physiological, picomolar concentrations of IL-15 cannot activate such target cells *in vivo*, this *trans*-presentation allows cells that do not express IL-15Rα to respond to physiological concentrations of IL-15. This phenomenon explains the intriguing but puzzling observations recently made by Averil Ma, MD's group (University of Chicago) that IL-15Rα expression on environmental cells is needed but expression on NK cells or CD8 memory T cells is dispensable (Lodolce JP et al. *J Exp Med* 194: 1187-94, 2001). Our data suggest the mechanism for their observation, which seems to contradict the prevailing theory.

Furthermore, we addressed which cell types express IL-15/IL-15Rα complexes and under what conditions. Interferons in conjunction with Toll-like receptor 4 stimulation induced the coordinated expression of IL-15 and IL-15Rα on monocytes and dendritic cells, indicating that activated antigen-presenting cells (APCs) are a source of membrane IL-15. Thus, IL-15/IL-15Rα complexes on APCs could be an important facet of the immunological synapse.

Our observation may also help resolve the paradox that two cytokines, IL-2 and IL-15, share β and γc receptor subunits and a common JAK1/3–STAT5 signaling pathway, yet manifest contrasting functions *in vivo*. IL-2 is a soluble factor, whereas IL-15 acts as a membrane-associated molecule *trans*-presented by APCs as part of an immunological synapse that also involves such other signals as T-cell receptor/peptide/major histocompatibility complex or CD28-CD80/CD86 complexes. Thus, the IL-15 signal can be more complex than that of IL-2.

In summary, our work presents two novel aspects of IL-15 biology, recycling and *trans*-presentation. On interaction with T cells, activated APCs may use IL-15/IL-15Rα complexes as a co-stimulatory factor through *trans*-presentation. We do not exclude a potential autologous role for IL-15Rα in the long-term survival of CD8 memory T cells; indeed, IL-15Rα expression on surviving CD8 memory T cells has been demonstrated (Berard M et al. *J Immunol* 170: 5018-26, 2003). T cells expressing IL-15Rα become resistant to apoptosis induced by IL-15 withdrawal, presumably because of the recycling of IL-15 associated with them. Therefore, the recycling element may promote long-term survival of CD8 memory T cells that express IL-15Rα.

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NCI Director Dr. Andrew von Eschenbach Visits the CCR

NCI Director Andrew von Eschenbach, MD, visited the CCR in February to tour building 37, which is undergoing renovation, and to learn more about the science being performed by our researchers. The 12th director of NCI since its creation in 1938, Dr. von Eschenbach took the helm of the organization in 2001 and quickly set an ambitious goal: to eliminate suffering and death due to cancer by 2015. Dr. von Eschenbach believes that because scientists now understand many of the mechanisms that cause a normal cell to become malignant, they can work together more effectively at finding ways to target and control cancer by intervening at the level of one or more of those mechanisms. Molecularly targeted and individualized therapies aimed at biologically controlling cancer represent a new paradigm in the battle against the disease such that if cancer cannot be completely eliminated, it can at least be managed as a chronic disease in less painful ways.

Achieving the goal of biologically controlling cancer involves a great deal of basic research, which is a strength of the CCR. Through their research, the CCR staff members have continued to work toward realizing this goal. During Dr. von Eschenbach's visit, several CCR investigators had an opportunity to discuss their research initiatives and findings with him. Specifically, three principal areas of science were presented: 1) noncoding RNAs as regulators of genes, 2) identification of signaling pathways disrupted in cancer and the targeting of specific pathway components, and 3) inflammation and energy balance in cancer development. The following is a brief discussion of some of the research presented to Dr. von Eschenbach.

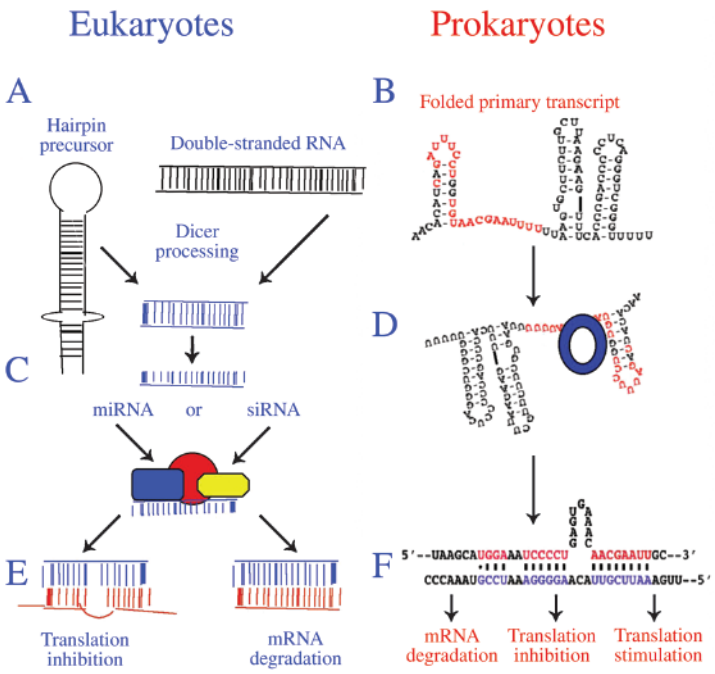

Figure 1. Both eukaryotes and prokaryotes use small RNAs as regulatory molecules. *A:* In eukaryotes, hairpin precursors encoded within the genome, or double-stranded RNA arising from repeated sequences or infections with RNA viruses, are processed to short double-stranded RNA molecules by a ribonuclease called Dicer. The product of the hairpins is called microRNA (miRNA). The product of double-stranded RNAs is called small interfering RNA (siRNA). *B:* In prokaryotes, the primary RNA transcript folds into an active form and does not require processing to act. *C, D:* In both prokaryotes and eukaryotes, the small RNA molecules must be bound to protein cofactors to act. *E, F:* After binding to the protein cofactor, the small RNA can interact by pairing with a target mRNA. *E:* In eukaryotes, if pairing is not precise, translation inhibition results. If pairing is complete, degradation of the target mRNA occurs. *F:* In prokaryotes, the outcome of pairing can be any of the fates shown, depending upon the location and properties of the paired complex. *(Figure and legend courtesy of Susan Gottesman, PhD)*

Noncoding RNAs as Regulators of Genes

Susan Gottesman, PhD, of the Laboratory of Molecular Biology, discussed her work in using *Escherichia coli* to develop methods to detect and define the function of small non-coding RNAs, which play important roles in stress responses and general metabolism via interactions with mRNAs and proteins. There are clear parallels between the modes of action of regulatory RNAs in *E. coli* and eukaryotic cells (Figure 1). In the future, these molecules may serve as powerful tools in developing novel approaches to cancer treatment and diagnosis.

Natasha Caplen, PhD, of the Gene Silencing Section, is investigating RNA-mediated silencing of gene expression in eukaryotes. In this mechanism, termed RNA interference, a double-stranded RNA structure is required to mediate a downregulation in gene expression through interaction of small RNA molecules (20–25 nucleotides) with a target RNA transcript. By way of this and similar research, highly effective RNA-based functional genomics tools are currently being developed with a potential for clinical application.

A Novel Oncofetal Gene and Smad7 as Potential Therapeutic Targets

David Salomon, PhD, of the Tumor Growth Factor Section of the Mammary Biology and Tumorigenesis Laboratory, is studying the potential role of *cripto-1* (*CR-1*), an oncofetal gene, in the development of cancer. Some evidence that cripto protein has a causative role in cancer relates to the fact that it is overexpressed in more than three quarters of human breast, colon, and lung cancers and more than half of testicular, stomach, pancreatic, and ovarian cancers and this overexpression promotes anchorage-independent growth, which is a marker for cellular transformation. Several anti-cripto monoclonal antibodies (MAbs) have been identified that may have tumor-suppressing activity (Figure 2A), possibly by suppressing angiogenesis (Figure 2B) (unpublished data).

Adam Glick, PhD, of the Laboratory of Cellular Carcinogenesis and Tumor Promotion, discussed his investigation

of Smad7 as a potentially useful target for cancer therapy. His work has produced the first data conclusively showing that Smad7 overexpression accelerates premalignant progression. Interestingly, cripto-1 is upregulated in cells overexpressing Smad7, and this may contribute to some of the altered differentiation properties of the tumor cells. A future goal is to use small interfering RNA technology to demonstrate that inhibition of Smad7 expression can block tumor progression, which will provide proof that Smad7 is a relevant molecular target for cancer therapy.

Physiologic Aspects of Cancer Development

Curtis Harris, MD, of the Laboratory of Human Carcinogenesis, discussed the integrative biological and translational research he is conducting to further understand chronic inflammation as a risk factor for many types of human cancer. Specifically, he is investigating the mechanistic interaction between nitric oxide and p53 as a crucial pathway in inflammatory-mediated carcinogenesis.

Stephen Hursting, MPH, PhD, and I described our study of the effects of energy balance modulation (obesity, diet, physical activity) on spontaneous tumor development, hormones, and gene expression in mice deficient in p53 or adenomatous polyposis coli (APC) protein tumor suppressor function. We are linking findings from basic research to clinical studies to develop more effective interventions for patients with cancer and obesity.

The research presented to Dr. von Eschenbach clearly reflects the key role that basic, translational, and clinical studies play in fighting disease. In particular, the use of noncoding RNAs as inhibitors of gene expression, a process that was identified in mammalian cells relatively recently in 2001, shows how basic research can be the progenitor of enormous practical applications, even in a short amount of time. Because no one knows where the new, most useful ideas will originate, a healthy balance must be maintained among basic, translational, and clinical areas of study. Many present

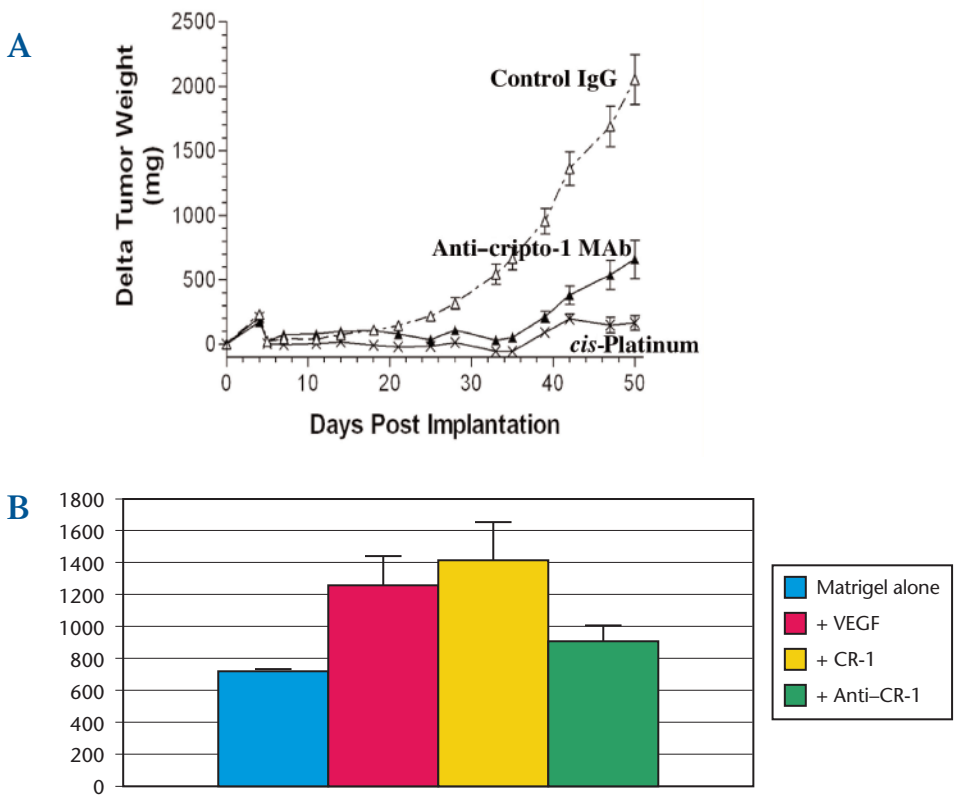

Figure 2. Inhibition of tumor growth (*A*) and inhibition of human endothelial cell angiogenesis by anti–cripto-1 monoclonal antibody (*B*). VEGF, vascular endothelial growth factor; CR-1, cripto-1 protein; anti–CR-1, anti–cripto-1 monoclonal antibody. *(Figure and legend courtesy of David Salomon, PhD)*

during the visit were impressed with Dr. von Eschenbach's ability to quickly relate basic science questions to clinical issues.

Removing Barriers

Dr. von Eschenbach's visit underscored two additional important ideas: informal communication and teamwork. The renovations being performed on the laboratory areas in building 37 are removing many of the physical barriers to informal communication, which facilitates the sharing of ideas among researchers, makes research more interactive, and evokes in investigators the feeling of being on a team. As Dr. von Eschenbach has said regarding this age in which collaboration, multidisciplinary partnerships, and an interdisciplinary approach are extremely important, "Individuals can excel, but teams will ultimately succeed."

Constantly sharing research results and building on past successes, the CCR investigators continue to make progress in delineating the molecular pathways of different cancers and in developing effective biologically based treatment modalities that may help to reduce or eliminate suffering and death. Moreover, the basic research being performed at the center has led to great strides being made in our understanding of other important biological processes that may also help investigators interfere with malignant pathways. Winning small battles at various points along the molecular routes of cancer may eventually help us to completely shut down all adverse roads, and win the greater war against this devastating disease.

■ **J. Carl Barrett, PhD**
Director

Special thanks to L. Michelle Bennett, PhD; Susan Gottesman, PhD; David Salomon, PhD; Natasha Caplen, PhD; Beverly Mock, PhD; Douglas Lowy, MD; Adam Glick, PhD; Curtis Harris, MD; Stephen Hursting, MPH, PhD; and Stuart Yuspa, MD

Novel Genetic Test for the Diagnosis of Cervical Dysplasia

Heselmeyer-Haddad K, Janz V, Castle PE, Chaudhri N, White N, Wilber K, Morrison LE, Auer G, Burroughs FH, Sherman ME, and Ried T. Detection of genomic amplification of the human telomerase gene (*TERC*) in cytologic specimens as a genetic test for the diagnosis of cervical dysplasia. *Am J Pathol* 163: 1405-16, 2003.

Morphological evaluation of cytological samples using Papanicolaou (Pap) smears or thin-layer preparations from cervical cell suspensions remains the pillar of cervical cancer screening. Such evaluation has greatly reduced the incidence of cervical carcinomas in countries that have implemented screening programs. Even so, a single cytologic examination is relatively insensitive, is poorly reproducible, and frequently yields equivocal results. Inadequate sampling, the scarcity of aberrant cells in some samples, and the subjectivity of morphologic interpretation are limitations, and false-negative evaluations account partly for the persisting incidence of cervical carcinomas (Koss LG. *JAMA* 261: 737-43, 1989). Additional, complementary tests are needed.

Most cervical cancers contain extra copies of the long arm of chromosome 3. Acquisition of these copies precedes the conversion of premalignant dysplastic cells to overt carcinomas. This finding was established several years ago (Heselmeyer K et al. *Proc Natl Acad Sci U S A* 93: 479-84, 1996; Heselmeyer K et al. *Genes Chromosomes Cancer* 19: 233-40, 1997); the role of this particular chromosomal aberration in the development of cervical cancer has since been confirmed. Chromosome 3 contains the gene that encodes the RNA component of human telomerase (TERC), a protein intricately involved in cell immortalization and cancer. We hypothesized that increased copy numbers of this gene contribute to cervical carcinogenesis and that visualization of gene copy number increases in diagnostic samples might be useful for the genetic diagnosis of cervical dysplasia (a premalignant precursor lesion of invasive cervical carcinomas).

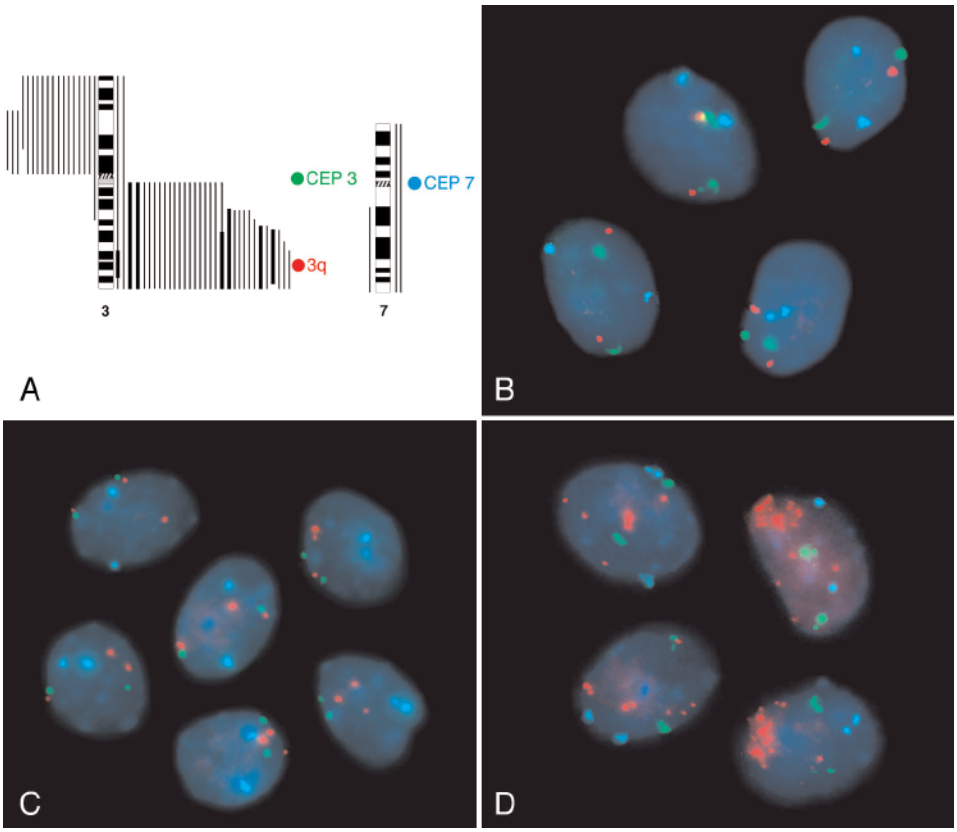

Figure 1. *A:* Triple-color probe set for the detection of chromosome 3q gain in cervical dysplasia. The probe set targets the chromosome 7 centromere (CEP7, labeled with Spectrum Aqua), the chromosome 3 centromere (CEP3, Spectrum Green), and the *TERC* gene (Spectrum Orange). The bars next to the ideogram show the comparative genomic hybridization results of 40 cervical carcinomas. *B:* The hybridization of the chromosome 3q probe panel to normal epithelial cells of the cervix uteri reveals two signals for each probe in all the nuclei. *C:* A high-grade squamous intraepithelial grade 3 lesion exhibited a variety of aberrant clones. Most cells showed three copies of the *TERC* gene in otherwise diploid cells. *D:* High-level amplification of *TERC* in a similar lesion.

We developed a three-color probe set to visualize TERC amplification in routinely ascertained Pap smears or thin-layer preparations. The objective visualization of chromosomal aneuploidies, such as copy number increases of chromosome 3q in the intact nuclei of cytological preparations, can be readily achieved using *in situ* hybridization with fluorescent DNA probes (Figure 1). In addition to a set of four overlapping bacterial artificial chromosome clones for the *TERC* gene, we included centromere-specific repeat probes that recognize chromosomes 3 and 7. We hybridized the probe cocktail to 57 routinely collected, rigorously screened, thin-layer slides classified as normal (n = 13), atypical squamous cells (ASC, n = 5), low-grade squamous intraepithelial lesions (LSIL, n = 14), or high-grade squamous intraepithelial lesions grade 2 (HSIL CIN2, n = 8) or grade 3 (HSIL CIN3, n = 17). All these samples were coded, and only after signal enumeration was the cytological diagnosis provided to us.

We found that 15.4% of normal, 0.0% of ASC, 7.1% of LSIL, 62.5% of HSIL CIN2, and 76.4% of HSIL CIN3 samples were

positive for extra copies of chromosome 3q (using 1% 3q-positive cells as the threshold based on the results of the hybridization to the normal peripheral blood lymphocytes). On average, 0.6% of normal, 0.1% of ASC, 0.3% of LSIL, 1.2% of HSIL CIN2, and 8.8% of HSIL CIN3 Pap smear samples showed additional 3q copy numbers.

The centromere-specific repeat probes allowed us to score the percentage of cells that had become tetraploid (four copies for all signals). In thin-layer specimens, 8% of normal, 0% of ASC, 29% of LSIL, 75% of HSIL CIN2, and 73% of HSIL CIN3 samples showed more than 1% tetraploid cells. Occasionally tetraploidy was observed even in the absence of copy number increases for chromosome 3q. In particular, the hybridization patterns observed for the HSIL CIN3 cases suggested that 3q amplification can occur in diploid cells or after genome tetraploidization.

Some cases revealed copy numbers of four or more for both centromere probes and numbers greater than four for the 3q probe. In other cases, however, the copy number of centromeres for chromosomes 3 and 7 remained two, and the copy number increases for the 3q probe varied. This finding suggested that amplification of TERC can occur without pre-existing genomic instability. The sensitivity and specificity of the signal enumeration test to discern high-grade lesions from lesser conditions (LSIL, ASC, and normal) both exceeded 90%.

In summary, detecting chromosomal aneuploidy and genomic amplification of the *TERC* gene allows for genetic diagnosis of cervical dysplasia independent of morphological assessment. The visualization of specific genomic markers of tumorigenesis in individual cells of routinely collected samples is particularly attractive for diagnosing early disease, a stage at which morphological changes are less pronounced and only a fraction of cells are actually aberrant. Our study was not designed to establish a causal relationship between gain of chromosome 3q and disease progression, but our findings hint that copy number increases of chromosome 3q are a necessary genetic alteration for cervical carcinogenesis, which, in cooperation with the action of high-risk human papilloma viruses, determines the "point of no return" in tumor progression.

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STRUCTURAL BIOLOGY

Specific DNA Recognition by the C/EBP Family of Transcription Factors

Miller M, Shuman JD, Sebastian T, Dauter Z, and Johnson PF. Structural basis for DNA recognition by the basic region leucine zipper transcription factor CCAAT/enhancer-binding protein α. *J Biol Chem* 278: 15178-84, 2003.

To control expression of their 30,000 or so genes, mammalian cells rely on DNA-binding transcription factors to recognize specific *cis*-regulatory DNA elements and thereby activate or repress gene transcription. Although three-dimensional structures of many protein-DNA complexes have helped identify several distinct DNA-binding motifs, no universal code relating the primary structure of a protein to its preferred DNA recognition sequence can be established. Indeed, closely related members from the same class of transcription factors often exhibit considerable differences in how they recognize DNA. This is true even for the simplest known DNA-binding motif, the basic region leucine zipper (bZIP) structure.

The prototypical bZIP protein, CCAAT/enhancer-binding protein α (C/EBPα), was discovered more than 15 years ago and was one of the first mammalian transcription factors to be purified and cloned. The canonical bZIP DNA-binding motif consists of a positively charged domain (the basic region) juxtaposed to a heptad repeat of leucine residues (the leucine zipper). The bZIP dimer was predicted to consist of two α helices held perpendicular to the DNA double helix, and dimerization to occur via the leucine zipper in the form of a coiled-coil. In this "scissors grip" model, each basic region forms a helical extension of the zipper α helix and contacts a half-site in the major groove of the DNA.

Although three-dimensional structures of many protein-DNA complexes have helped identify several distinct DNA-binding motifs, no universal code relating the primary structure of a protein to its preferred DNA recognition sequence can be established.

The known bZIP proteins now comprise several subfamilies (C/EBP, AP-1, PAR, CREB/ATF, PAP, and plant GBF), classified according to their DNA sequence specificities. X-ray structures of several bZIP-DNA complexes revealed that bZIPs recognize specific DNA sites through base contacts

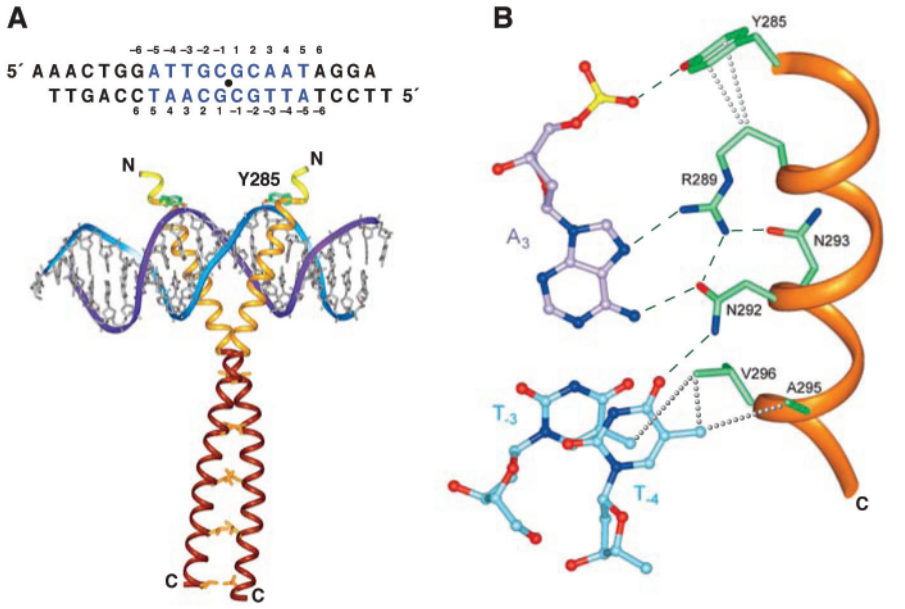

Figure 1. Crystal structure of the CCAAT/enhancer-binding protein α (C/EBPα) basic region leucine zipper (bZIP) domain bound to its cognate DNA. *A:* Overall view of the complex. The sequence of the DNA duplex used for crystallization is shown at the top, with the consensus C/EBP site indicated in blue; numbers indicate nucleotide positions. The protein is shown as a ribbon, with the basic region in yellow and the leucine zipper in brown. N, amino terminus; C, carboxy terminus. *B:* Critical interactions in the C/EBPα protein-DNA interface. Protein chains are represented as sticks, and DNA is shown as balls and sticks. Dashed lines, important electrostatic interactions; dotted lines, van der Waals interactions.

made by five residues within the basic region motif characteristic for each subfamily. However, basic regions are quite similar among all bZIP proteins and contain two invariant residues, asparagine and arginine, raising the question of how different bZIP proteins recognize their cognate DNA sites while discriminating against related sequences.

We have recently determined the 2.8-Å crystal structure of the C/EBPα bZIP domain bound to its DNA ligand (Figure 1A). The general structure conforms to those observed for other bZIP proteins. Important details of DNA recognition, however, emerge on close inspection of the protein-DNA interface. Direct contacts with DNA are made by Arg289, Asn292 (invariant in bZIPs), Ala295, Val296, Ser299, and Arg300. A striking feature is the central role of Arg289, which is hydrogen bonded to base A_3, phosphate, Asn292, and Asn293 (Figure 1B) and is conformationally restricted by Tyr285. Accordingly, mutation of Arg289 to Ala289 completely abolished C/EBPα DNA-binding activity. Members of the Jun/Fos family have an analogous arginine residue, but in these proteins the side chain adopts a different conformation and makes no contact with DNA bases. Thus, the essential functional role for Arg289 in C/EBPα could not have been predicted from structures of other bZIP proteins, nor was it identified in previous mutagenesis studies.

Another critical residue is Val296. Substituting Ala296 for Val296 dramatically increased C/EBPα's affinity for cyclic AMP response element (CRE) sites but did not reduce binding to C/EBP sites. Hence, Val296 contributes to C/EBP DNA-binding specificity mainly by restricting interactions with closely related sequences like CREs.

One of the biological roles of C/EBPα is to induce cell growth arrest during terminal differentiation. A recent study predicted that Tyr285 in the basic region mediates an association between C/EBPα and the cell cycle transcription factor E2F, and that this interaction is required for C/EBPα to induce cell cycle arrest (Porse BT et al. *Cell* 107: 247-58, 2001). The phenotype of "knock-in" mice carrying the C/EBPα Tyr285Ala point mutation was similar to, but less severe than, the phenotype of mice with a complete knockout of C/EBPα. The crystal structure now shows that Tyr285 is an integral component of the protein-DNA interface, and mutation of this residue strongly decreased the affinity of both C/EBPα and C/EBPβ for DNA. Thus, the phenotypic effects of mutating Tyr285 can be explained by reduced DNA-binding activity of the mutant protein, and the structural data do not directly support a role for this residue in E2F interactions.

The C/EBPα crystal structure complemented with mutagenesis has revealed the basis for specific DNA recognition by C/EBPα. Because residues that participate in the C/EBPα-DNA interface are identical throughout the C/EBP family, the same DNA-recognition mechanism should apply to other C/EBP proteins. The structural information now available may also be useful in predicting which residues from C/EBP bZIP regions participate in associations with other cellular proteins. With recent work implicating both C/EBPα and C/EBPβ in tumorigenesis (Pabst T et al. *Nat Genet* 27: 263-70, 2001; Zhu S et al. *Proc Natl Acad Sci U S A* 99: 207-12, 2002), structural characterization of other functional domains in these proteins is an important future goal.

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Spindle Checkpoint Proteins "Mark" Chromosomes in Trouble

Kerscher O, Crotti LB, and Basrai, MA. Recognizing chromosomes in trouble: association of the spindle checkpoint protein Bub3p with altered kinetochores and a unique defective centromere. *Mol Cell Biol* 23: 6406-18, 2003.

Chromosome segregation—the process of controlled separation and movement of chromosomes from the mother to the daughter cell—requires many cellular components, including spindle pole bodies, which are connected via spindle microtubules to each chromosome's kinetochore (a complex of centromere [*CEN*], DNA, and associated proteins). The maintenance, replication, and segregation of chromosomes are controlled by the mitotic cell cycle. During this cycle, checkpoint proteins provide a fail-safe mechanism for faithful chromosome segregation by allowing extra time for chromosomes to properly align. The interplay of at least six evolutionarily conserved spindle checkpoint proteins (Mad1p, Mad2p, Mad3p, Bub1p, Bub3p, and Mps1p) monitors the integrity of the kinetochore/microtubule complex and mediates a "halt anaphase" signal even if only a single chromosome's association with a microtubule is altered or compromised (Musacchio A et al. *Nat Rev Mol Cell Biol* 10: 731-41, 2002).

Until recently, the majority of *in vivo* cell biological observations pertaining to checkpoint proteins have been made in multicellular eukaryotic cells, which are not easily amenable to genetic analysis. Thus, we undertook a comprehensive analysis of the *in vivo* localization and biochemical associations of Bub3p in the context of its functional role in checkpoint pathways in *Saccharomyces cerevisiae* by using Bub3p tagged at its C-terminal end with green fluorescent protein (GFP) (Bub3-GFP).

During the unperturbed cell cycle, we observed distinct nuclear Bub3-GFP foci that overlapped with a subset of foci corresponding to the kinetochore protein Mtw1p but not with spindle pole body proteins Spc29 or Bub2p. We tested whether alterations or defects in kinetochore/spindle integrity lead to the enrichment of Bub3p foci. In the first of such experiments, we examined the localization and biochemical properties of Bub3p during spindle checkpoint activation using nocodazole (NOC)—which induces spindle disassembly. Chromatin immunoprecipitation (ChIP) experiments showed that in cells arrested with NOC, Bub3-GFP associates specifically with *CEN* DNA and no other tested loci. We also determined that spindle checkpoint protein Bub1p and kinetochore protein Ndc10p are required for the kinetochore association of Bub3-GFP. Our results are consistent with a similar requirement of Bub1p for the kinetochore association of Bub3-GFP in *Drosophila melanogaster* and *Xenopus laevis* (Basu J et al. *Chromosoma* 107: 376-85, 1998; Sharp-Baker H et al. *J Cell Biol* 153: 1239-50, 2001), suggesting that the functional interaction between Bub3p and Bub1p is likely conserved from yeast to higher eukaryotes.

Under normal physiological conditions, a cell would rarely, if ever, encounter a

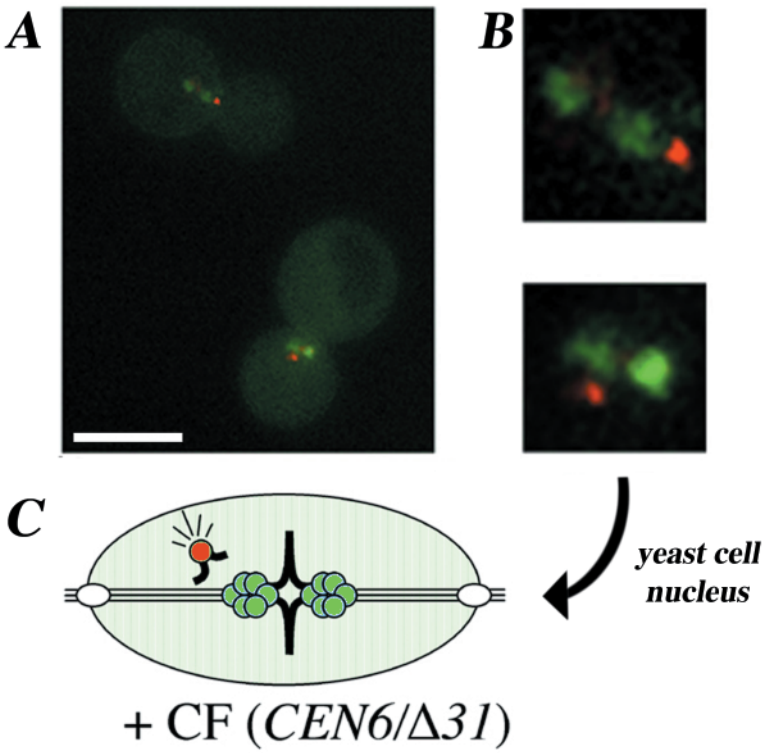

Figure 1. Bub3p associates with a single defective centromere in *Saccharomyces cerevisiae. A:* Image depicts a mitotic cell with clustered bipartite kinetochores (enlarged in part *B*) that are labeled with Mtw1 tagged with cyan fluorescent protein (CFP) (green) and Bub3p tagged with green fluorescent protein (GFP) (Bub3-GFP) (red) in the reporter strain with a chromosome fragment (CF) carrying the defective centromere element *CF/CEN6(Δ31).* (Scale bar in white represents 5.0 μm.) The Bub3-GFP foci exist as separate entities away from the main kinetochore cluster. In *S. cerevisiae,* the 16 kinetochores cluster and are observed as one mass before mitosis. *C:* A model for *in vivo* association of Bub3-GFP with altered kinetochores. Inside the nucleus (large oval) are kinetochores (small green spheres) of chromatids that are tethered via microtubules (|||) to the spindle pole bodies (SPB) (small white ovals) in the nuclear envelope. Association of Bub3-GFP with the kinetochore from the single defective CF (red sphere) suggests a preferential association with defective kinetochore/spindle structures under conditions that activate the spindle checkpoint and delay in mitosis (\ | /). The enrichment of checkpoint protein(s) at kinetochores may provide the "halt anaphase" signal.

situation in which all of the chromosomes have a defect in kinetochore/spindle integrity analogous to NOC treatment. It seems more likely that during mitosis, the interaction of a single kinetochore/spindle is compromised, triggering spindle checkpoint activation. How can we probe the interaction of a checkpoint protein like Bub3p with a single defective kinetochore?

We used the awesome power of yeast genetics, cell biology, and biochemistry to examine the localization of Bub3-GFP in a novel reporter strain containing a single defective centromere in the context of 16 normal centromeres. The defective centromere contained a deletion of a 31-base-pair region in the central *CEN6* element, CDEII or *CEN6(Δ31)*, which is carried on a non-essential chromosome fragment (CF). The *CEN6(Δ31)* mutation leads to a high rate of loss of this CF and a delay in mitosis (Spencer F et al. *Proc Natl Acad Sci U S A* 89: 8908-12, 1992). In the reporter strain, we observed an increased number of smaller-sized, sharply delineated Bub3-GFP foci that showed little or no overlap with the kinetochore marker Mtw1 (Figure 1). Quantitative ChIP experiments confirmed that Bub3-GFP preferentially associated with defective kinetochores *in vivo*. Therefore, it is possible that an unattached *CF/CEN6(Δ31)* is recognized by Bub3-GFP and the resulting foci "mark" the defective CF for possible retrieval prior to anaphase.

Our data are consistent with the molecular role of the checkpoint proteins previously studied via genetic and *in vitro* biochemical analyses in *S. cerevisiae*. Our studies, however, represent the first comprehensive analysis of spindle checkpoint protein behavior in the presence of a single defective kinetochore in the context of wild-type kinetochores by using live cell imaging and the ChIP technique. The reporter strain with a single defective centromere can be used to identify proteins required for the assembly of spindle checkpoint complexes in yeast and other systems. These studies are particularly important as mutations in checkpoint genes lead to chromosome instability in yeast (Warren C et al. *Mol Biol Cell* 13: 3029-41, 2002). Furthermore, in humans, some cancers displaying a chromosomal instability (CIN) phenotype show loss of function of Bub1p, which interacts with Bub3p and is required for the association of Bub3p with kinetochores (Cahill DP et al. *Nature* 392: 300-03, 1998). Understanding the molecular role of checkpoint proteins will thus contribute greatly to the study of aneuploidy, cancers, and developmental catastrophes. Ultimately, we would like to know if preferential enrichment of checkpoint proteins can be a useful diagnostic tool to detect genetic instability.

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CLINICAL RESEARCH

The Multidisciplinary Head and Neck Cancer Clinical Research Program

This year, more than 42,000 Americans will develop cancers of the upper aerodigestive tract (head and neck, larynx and esophagus) and more than 12,000 of these patients will die of their disease. Many patients in the United States will suffer significant impairment of their speech or other major disabilities as a result of their tumors or as complications of their treatment. NIH physician-scientists are investigating predictors that can elucidate the course of malignant lesions of the head and neck, as well as guide therapy for these cancers, most of which come to medical attention at an advanced stage. New strategies for prevention and therapy are being studied to preserve organ function, reduce patient disability, and improve patient survival. The complexity of these diseases lends itself to a multidisciplinary treatment approach. Close collaboration between laboratory and clinical scientists and patients is needed to develop more effective therapies.

The Multidisciplinary Head and Neck Cancer Group at the NIH has ongoing clinical trials and studies in development for patients with locally advanced, recurrent, and metastatic cancer of the head and neck. Collaborations with other NIH investigators and with researchers outside of the NIH allow the team to recommend innovative, state-of-the-art therapy for patients with malignancies affecting the head and neck. Screening of high-risk patients, consultations, and "second opinion" services are also provided.

The ongoing clinical trials include the following:

- Radiation and bortezomib (Velcade) combined modality treatment for recurrent squamous cell carcinoma of the head and neck—this trial builds on the preclinical work performed at the NIH showing that bortezomib enhances radiation, inhibits head and neck cancer cell growth and new blood supply formation, and inhibits NFκB, a survival factor often produced in cancer cells by radiation and chemotherapy.
- Paclitaxel, gefitinib (Iressa), and radiation combined-modality treatment for untreated locally advanced or unresectable squamous carcinoma

of the head and neck—this trial builds on a successful platform of taxane-radiation previously studied at the NIH. Gefitinib inhibits the epidermal growth factor receptor and subsequently other survival factors that may be induced by radiation therapy.

- Flavopiridol for metastatic/recurrent squamous cell carcinoma of the head and neck—this trial is evaluating a novel cell-cycle inhibitor for activity in squamous cancers of the head and neck.

The Multidisciplinary Head and Neck Cancer Clinical Research Team represents several specialties across three institutes within the NIH:

- The National Cancer Institute (NCI): Barbara Conley, MD; John Morris, MD; and Dimitri Colevas, MD—Medical Oncology; David Gius, MD, PhD—Radiation Oncology.
- The National Institute for Deafness and Other Communicative Disorders (NIDCD): Carter VanWaes, MD, PhD and Susan Rudy, NP.
- The National Institute for Dental and Craniofacial Research (NIDCR): Adrian Senderowicz, MD; Jaime Brahim, DDS; and Ramin Razavi, DDS.
- Rehabilitation Medicine/Speech and Swallowing: Beth Solomon, CCC-SLP—NIH Clinical Center.

For information on studies, arranging consultation visits, or other questions, please contact the following individual:

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ADMINISTRATIVE LINKS

CCR Intranet: Useful Links and Information
CCR's intranet home page (http://ccrintra.cancer.gov) offers principal investigators and other scientific staff many useful links and important information. There are guidelines and templates for upcoming site visits, listserves for the NCI faculties and working groups, a link to the Institutional Review Board (IRB) website, patent and invention information, and media guidelines and tips. There are also links to archived issues of *CCR Frontiers in Science* and the *60-Second Update*.

New NCI "Express Services" Website
If you work on the Bethesda campus, you can now make service requests online. The NCI Office of Space and Facilities Management has just launched a new website called "Express Services," to assist employees in requesting handyman services for such things as painting, carpeting, locks, keys, carpentry, electrical problems, as well as hanging pictures, clocks, plaques, etc. To submit a request, just go to http://camp.nci.nih.gov/admin/osfm/express.html and enter your NCI username and password. All NCI-Bethesda employees are encouraged to submit requests through this new website or to contact their respective Administrative Officer for assistance.

NCI Administrative Intranet: A New Tool for Administrative Staff
The new NCI Administrative intranet website has been launched and can be viewed at http://camp.nci.nih.gov/admin/index.html. Specifically developed for NCI administrators and administrative support staff, this site contains links/information on a variety of useful resources, such as NCI management offices, minutes from NCI Intramural Advisory Board meetings, standard NIH forms and policies, and training.

LION: The NCI Library Online
Introducing LION, a joint project of the NCI Library and the NCI Office of Communications. LION is an online repository and alerting service for NCI electronic resources, books, journals, archival materials, and Internet links and content. One of the main goals of LION is to make these resources as widely accessible as possible within the Institute. NCI staff members can browse, search, and request materials from this extensive collection, which also includes articles, press releases, pamphlets, and audio/visuals. To visit the LION site, go to https://lion.nci.nih.gov.

NIH Telework Website
Thinking about telework as an alternative? If so, then check out the NIH Telework website at http://telework.od.nih.gov. This site contains information and links related to NIH's telework policy, application and agreement forms, resources, and frequently asked questions. There are also individual success stories posted to help those who are considering telework as an alternative workplace.

Thomas Hornyak, MD, PhD

Thomas Hornyak, MD, PhD, is a native of Temperance, Michigan. He received his AB in music from Princeton University in 1985, then entered the MD/PhD program at the University of Michigan Medical School. He received his PhD in biological chemistry, having performed his doctoral work in the laboratory of Jules A. Shafer, PhD. His research at that time involved the use of enzyme and chemical kinetics to study factors modulating the activation of blood coagulation factor XIII.

After graduation in 1992, he completed an internship in medicine at the New York Hospital–Cornell University Medical Center and a residency in dermatology at New York University (NYU) Medical Center. While a dermatology resident at NYU, he became interested in melanocyte developmental biology and its relationship to human pigmentary disorders. He began working on melanocytes in the laboratory of Edward B. Ziff, PhD in the NYU Department of Biochemistry, studying transcription factors regulating melanocyte development during embryogenesis and the regulation of the melanogenic enzyme gene *Dct*, which encodes dopachrome tautomerase/tyrosinase-related protein-2.

In 1999, Dr. Hornyak joined the Department of Dermatology at the Henry Ford Health System as a staff member and independent investigator. At Henry Ford, he continued his previous work and initiated a project on neurofibromin in melanocyte differentiation. He organized and directed a pigmented lesion clinic, treating many patients with melanoma and melanocytic nevi, and received the Edward A. Krull Faculty Role Model Award given by the dermatology residents. He has received several extramural NIH research grants and a New Investigator Award from the Department of Defense Neurofibromatosis Research Initiative.

In August 2003, Dr. Hornyak joined the Dermatology Branch of the CCR as a tenure-track investigator. His group works on how specific transcription factors and signal transduction pathways regulate normal melanocyte proliferation, differentiation, and survival during development. A new interest of his group at CCR is investigating how dysregulation of these pathways, especially resulting from somatic oncogene mutations characteristic of human melanomas and premalignant melanocytic lesions, contributes to aberrant cell growth, migration, and survival in malignant melanoma.

Dr. Hornyak

Dr. Hornyak and his wife Mary live in Bethesda, MD with their children Mark, age 6, and Cecilia, age 3. In his spare time, he enjoys family outings and playing the piano.