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FROM THE DIRECTOR

Cancer Redox Biology Faculty Calls a Workshop

Several oxidation-reduction mechanisms are important in cancer etiology and treatment. There is so much to learn about nitric oxide (NO) and its oxidation-reduction reaction, called redox, that David Wink, PhD, of the NCI CCR Radiation Biology Branch, formed the Cancer Redox Biology (CRB) Faculty to do just that. The Faculty has successfully enhanced communication and promoted collaboration among biochemists, chemists, clinical oncologists, epidemiologists, and others interested in the molecular mechanisms by which redox stress alters cancer development and tumor spread.

The CRB Faculty recently assessed the state of the science in a workshop on "Redox-Based Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in Cancer Treatment, Prevention and Angiogenesis: A Novel Solution to an Old Problem." It was, in part, a response to an extensive hearing by the U.S. Food and Drug Administration (FDA) in 2005 that suggested the entire class of NSAIDs might have problematic side effects.

Dr. David Wink of CCR's Radiation Biology Branch *(right)* presents Nobel Laureate Dr. Louis Ignarro (UCLA) with a lifetime achievement award at the Grand Rounds Lecture at the workshop.

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A few years before, researchers had claimed that NSAIDs such as aspirin could inhibit cyclooxygenase (COX) enzymes and help prevent colorectal cancer. The oncology community soon discovered that aspirin causes side effects such as stomach ulcers. Renewed optimism followed with the discovery of selective COX-2 inhibitors, such as celecoxib or rofecoxib, which targeted COX-2 exclusively, reducing prostaglandin (PG) E_2 levels in cells without causing stomach toxicity. However, these new agents also brought new side effects.

The workshop assessed NSAIDs from a CRB perspective. The day opened with Dr. Louis Ignarro, Nobel Laureate in Medicine from the David Geffen School of Medicine at the University of California, Los Angeles, delivering the Grand Rounds Lecture. He presented his exceptional discoveries that clarified the role of NO as a unique signaling molecule beneficial for patients who have heart disease.

The CRB Faculty and invited speakers then shared some of the translational research aimed at the COX-2 target. NO-aspirin, a potential new drug to inhibit COX-2 enzymes with NO covalently attached, drew much attention from the scientists. Data presented by Basil Rigas, MD, DSc, of the Cancer Prevention Division of the State University of New York at Stony Brook, showed that NO-aspirin was over 1,000-fold more potent than aspirin at inhibiting colon cancer cellular proliferation. And in animal models of colon and pancreatic cancer, it reduced tumor proliferation with little toxicity. Novel NSAIDs such as this may eventually provide more treatment options to patients with adenocarcinomas in breast and lung tissues.

Nitrogen Redox Family

Compound		*Oxidative State*
Nitrate	NO_3	+5
Nitrite (nitroxide)	NO_2	+3
Nitric oxide	NO	+2
Nitroxyl	NO^-	+1
Nitrogen	N_2	0
Hydroxylamine	NH_2OH	-1
Ammonia	NH_3	-3

Other researchers showed that the selective COX-2 inhibitor celecoxib is proving effective in lung cancer treatment. In a phase II clinical trial, celecoxib increased the survival of lung cancer patients compared with those who received chemotherapy alone. In a phase I study, again treating lung cancer, this agent used in combination with radiotherapy increased patient response more than radiation alone.

Sulfur (S)-NSAIDs were evaluated as well. Piero del Soldato, PhD, of CTG Pharma in Milan, Italy, reported that the S-NSAIDs showed little gastrointestinal toxicity. Grace Yeh, PhD, and David Roberts, PhD, described their important properties with respect to cancer prevention and treatment. Larry Keefer, PhD, presented chemical delivery systems that can target NO to specific sites within the body.

The CRB Faculty is an excellent example of successful multidisciplinary teaming within the CCR that leverages existing resources to support translational research. The success of this Faculty stems from a well-defined mission, strong leadership, and excellent communication among the members. Much preclinical work on NO remains to be done, but based on the pace of the CRB Faculty to date, this work will be accomplished soon and used to guide researchers in their selection of lead compounds to take forward to clinical trials.

■ **Robert H. Wiltrout, PhD**
Director

■ TUMOR BIOLOGY

Axon Guidance Cues in Tumor and Developmental Angiogenesis

Bedell VM, Yeo S-Y, Park KW, Chung J, Seth P, Shivalingappa V, Zhao J, Obara T, Sukhatme VP, Drummond IA, Li DY, and Ramchandran R. *roundabout4* is essential for angiogenesis *in vivo*. *Proc Natl Acad Sci U S A* 102: 6373–8, 2005.

Three decades of angiogenesis research has culminated with the first U.S. Food and Drug Administration (FDA)–approved anti-angiogenic drug, Avastin, which is showing promise in the clinic. Avastin, a monoclonal antibody to vascular endothelial growth factor (VEGF), sops up VEGF secreted by tumors and thus denies them the ability to grow new blood vessels. However, VEGF has other physiological functions, which are compromised in Avastin-treated patients. Because of this, discovering novel targets of vascular endothelium has taken on a new sense of urgency and prompted the search for the next generation of anti-angiogenic drug targets that are tumor-vasculature specific. Cell-surface moieties are of particular interest; historic evidence suggests that they might be suitable as targets.

Hints for such targets have unexpectedly come from the developmental patterning mechanism for the nervous system. Increasing evidence is emerging that similar patterning mechanisms are used during the development of neural and vascular networks in vertebrates. Molecules that guide axons to their targets appear to have counterparts that guide the patterning of vessels. Four families of guidance molecules, namely netrins, semaphorins, ephrins, and slits, and their cognate receptors, mediate axon guidance. Each of these guidance families has recently been shown to contain at least one ligand-receptor pair that plays a functional role in patterning of the vasculature. Our study focuses on the slit-robo signaling system in vascular development and investigates one member of the Roundabout (Robo) family, robo4, in vessel guidance.

Robos were originally found in *Drosophila* to mediate repulsive cues for slit ligands by preventing the re-crossing of axons once they have crossed the midline. Four members of the Robo family have been identified. Robo1, robo2, and robo3 are primarily expressed in the central nervous system and help guide axons. Outside the nervous system, robos have been thought to play a role in leukocyte trafficking and kidney branching morphogenesis. However, until the discovery of the fourth member of this family, robo4, and its expression patterns in developing mouse vasculature, robos were unappreciated in vascular development. Besides developmental angiogenesis, robo4 is expressed in sites of active angiogenesis in tumor vessels. Because robo4 is both selective to tumor vasculature and is a cell-surface receptor, it is an attractive target for tumor endothelium.

To investigate robo4 function, we studied its ortholog in zebrafish during embryonic development. We characterized *robo4* gene expression and pursued functional studies by gene knockdown approaches using morpholinos (antisense oligonucleotides). Robo4's *in situ* expression showed three interesting features. First, its expression in the embryonic zebrafish vasculature was transient and was seen in both angioblasts (Figure 1, parts A and B, white asterisk) and intersomitic vessels (ISVs) (Figure 1, parts C and D, black asterisks). Second, robo4 expression in notochord and ISVs of the trunk region overlapped, such that a concomitant decrease in robo4 expression in notochord in a rostral-to-caudal manner was immediately followed by expression in ISVs, suggesting a mechanistic role for robo4 in the ISV sprouting process (Figure 1, parts B and C). Third, robo4 expression in rostral sprouts ceased first, prior to its expression in caudal sprouts, suggesting a temporal regulation for the ISV sprouting process. To assess phenotype, we performed *in situ* studies with both endothelial (flk) and neuronal (acetylated tubulin) markers in *robo4* knockdown embryos. Compared with wild-type embryos (Figure 1, part E), *robo4* knockdown embryos displayed either a lack of ISV sprouts (Figure 1, part F) or linear ISV sprouts, albeit weak ones in the zebrafish trunk region.

To visualize the ISV vessel growth in a live embryo, we pursued time-lapse imaging of the trunk vasculature in embryos injected with morpholinos. Using live time-lapse imaging in *robo4* knockdown vascular-specific transgenic embryos, we demonstrated that when vessel sprouting occurs incorrectly, vessel formation is often aborted. Also, embryos often displayed a loss of temporal and spatial regulation of ISV sprouting from the dorsal aorta. The surprising finding was that prior to our gene knockdown study, it was widely assumed that robos were primarily negative regulators of guidance; however, our study suggests that removal of a presumptive negative guidance cue resulted in the collapse of vessels as opposed to the expected increase in sprouts. This result suggests that in the absence of guidance molecules, vessels normally have a default mechanism that prevents them from growing into incorrect tissue sites. This helps clarify why robo4 would be upregulated in tumor vasculature since, from a tumor standpoint, a cancer cell would subvert or override normal developmental guidance checkpoints and utilize this mechanism to form a chaotic vascular network, which is seen routinely in tumor vasculature. Another interpretation of our knockdown result is that besides traditional repulsive cues, robos mediate attractive cues as well. In fact, preliminary unpublished evidence from our laboratory suggests that this cannot be excluded, and perhaps, tumors use axon guidance molecules, which are known to have bifunctional properties, to fulfill their growing needs.

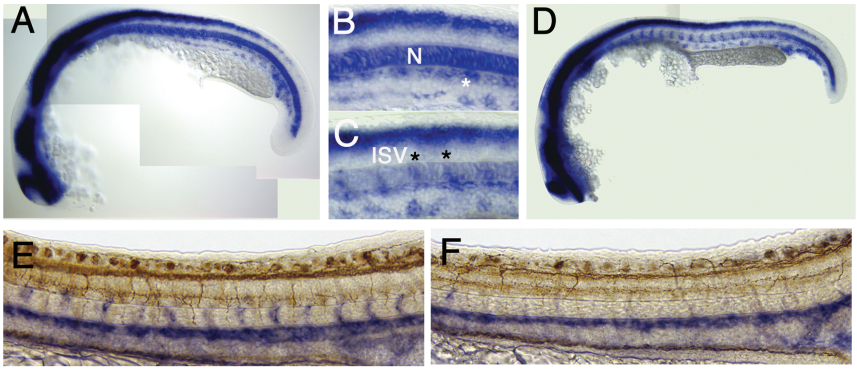

Figure 1. Robo4 expression patterns in embryonic zebrafish vascular development. Panels A through D show robo4 *in situ* (blue) across 20 *(A)* and 22 *(D)* somites in embryos. Panels B and C are high-power images of the trunk region of *(A)* and *(D)*, respectively. *(E)* Wild-type embryo and *(F)* morpholino-injected embryo. Panels E and F depict trunk regions of 22-somite embryos double stained for *flk* RNA (blue, endothelial marker) and antibody to acetylated tubulin (brown, neuronal marker). N, notochord; ISV, intersomitic vessels. Black asterisks depict the location of ISVs, and the white asterisk depicts angioblasts.

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From T-Cell Antigen Receptor Engagement to Cytoskeleton Reorganization

Barda-Saad M, Braiman A, Titerence R, Bunnell SC, Barr VA, and Samelson LE. Dynamic molecular interactions linking the T-cell antigen receptor to the actin cytoskeleton. *Nat Immunol* 6: 80–9, 2005.

Once the T-cell receptor (TCR) binds foreign antigens on antigen presenting cells (APCs), multiple proteins redistribute to form the immunological synapse. Organization of the synapse and the creation of a tight seal between the T cell and APC depend on actin polymerization. Studies using biochemical and imaging techniques have shown the formation of signaling assemblies at the TCR; however, studies of the molecular interactions linking the TCR to the cytoskeleton proteins in live cells have been rare.

In the present study, we focused on the dynamic interactions that occur between signaling molecules crucial for translating TCR engagement into localized actin polymerization. This goal was accomplished by using advanced molecular imaging techniques to observe T-cell spreading at the level of single activated T cells. During these events, we characterized the dynamic localization of the proteins regulating actin polymerization and demonstrated the molecular interactions involved in this process (Figure 1). We defined protein-protein interactions by performing quantitative characterization at the Angstrom level using fluorescence resonance energy transfer (FRET) techniques.

Our studies demonstrated that actin polymerization began at the site of TCR engagement and then migrated to the cellular periphery. Actin polymerization was driven by the Wiskott-Aldrich Syndrome protein (WASp) and was dependent on its dynamic localization. We demonstrated the critical role of TCR-induced tyrosine phosphorylation of the adaptor proteins LAT and SLP-76 in recruiting other adaptors, Nck and WASp, that are required for actin polymerization. The use of mutant T-cell lines lacking LAT or SLP-76 revealed a complexity in the mechanism of actin polymerization. In LAT-deficient cells, Nck and WASp did not cluster at the TCR. Nck also failed to cluster at the TCR in SLP-76–deficient cells. Reconstitution of these cells with the appropriate cDNA (LAT or SLP-76) restored normal recruitment of the tagged molecules at the TCR. However, reconstitution of LAT-deficient cells with a mutant form of LAT lacking four critical tyrosine residues, the sites of important phosphorylation, failed to reconstitute Nck recruitment. In the deficient cells, analysis of actin status revealed significant, albeit incomplete, inhibition of polymerization.

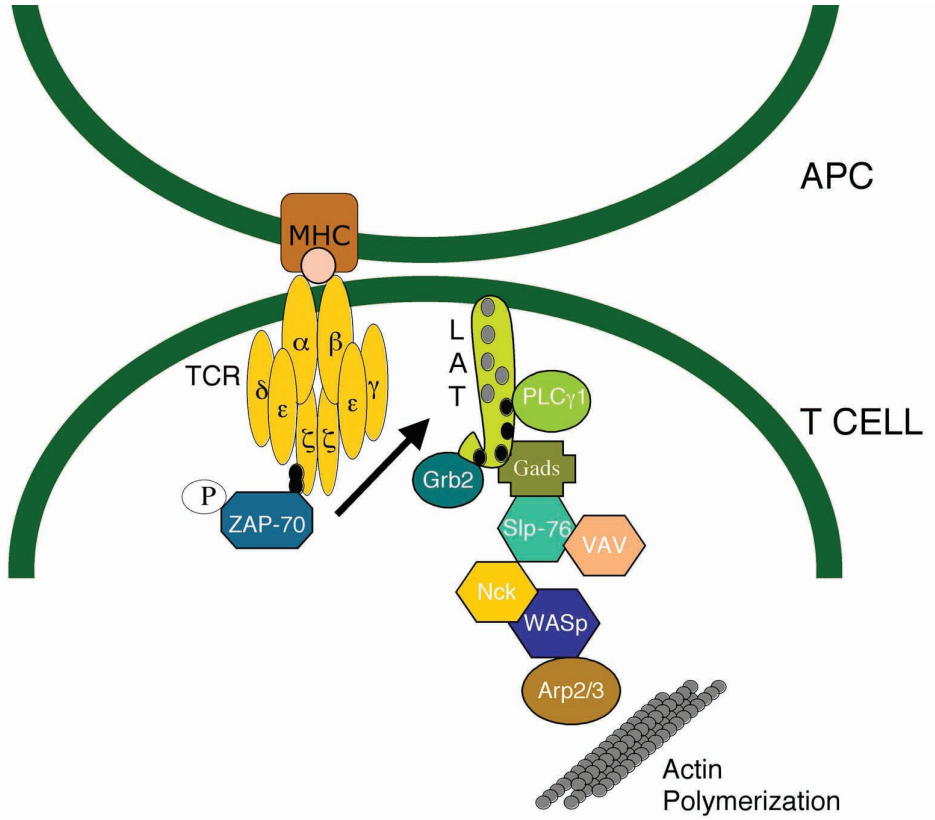

Figure 1. Molecular interactions leading to T-cell receptor (TCR)–induced actin polymerization. T-cell activation is initiated by antigen presenting cells (APCs) containing stimulatory major histocompatibility complex (MHC)–peptide complexes. Phosphorylation of the TCR (black circles) is mediated by Src family protein tyrosine kinases. ZAP-70 is recruited to the phosphorylated TCR subunits through its SH2 domain. It is phosphorylated and activated by the Src family protein tyrosine kinases. The linker for the activation of T cells (LAT) is tyrosine phosphorylated by ZAP-70. LAT contains nine tyrosine residues that, when phosphorylated, act as docking sites for adapter proteins such as Grb2 and Gads. SLP-76 is recruited by the LAT-nucleated complex through its interaction with the SH3 domains of Gads. SLP-76 associates with the SH2 domain of Nck. Nck binds WASp, which in turn binds the Arp2/3 complex that mediates actin polymerization.

The role of Nck in T-cell antigen receptor action has recently received much attention. Gil et al. (*Cell* 109: 901–12, 2002) reported experiments indicating that Nck directly binds TCR/CD3ε chains upon TCR engagement due to induced exposure of proline-rich sequences within the CD3ε. Recruitment of Nck to CD3ε was found to precede phosphorylation of it and other TCR subunits. Also, this binding was found to be independent

of SLP-76. The cellular reagents used in our current study enabled us to test these observations. Two of our results contradicted the study. First, the Src kinase inhibitor PP2 was used to block proximal tyrosine phosphorylation. Despite TCR-induced clustering, no activated ZAP-70, SLP-76, or Nck was detected in clusters at the cell membrane. We attribute this failure of recruitment to the lack of TCR phosphorylation induced by Lck or Fyn, the consequent lack of ZAP-70 recruitment and activation, followed by the failure of any ZAP-70 SH2—mediated interactions.

Since our means of activation is via CD3ε-mediated clustering of TCRs, the failure of Nck recruitment argues against recruitment being dependent on CD3ε conformational changes exposing Nck binding sites. Also, the expression of YFP-Nck in the SLP-76—deficient cells was used to test whether SLP-76 is necessary for Nck recruitment. In the absence of SLP-76, Nck was not recruited to the TCR, whose activation was confirmed by the presence of pZAP-70 at the TCR. Restoration of Nck clustering in these cells was demonstrated following reexpression of SLP-76. Thus, Nck recruitment in our studies requires tyrosine phosphorylation and SLP-76.

Our studies demonstrated that actin polymerization began at the site of TCR engagement and then migrated to the cellular periphery.

To confirm that the same protein recruitment and interactions occur in normal, non-transformed cells, we adapted the assay to human peripheral blood lymphocytes. Though the kinetics were slower, the pattern of protein localization in these cells was similar to that observed with Jurkat cells. Nck and WASp were initially recruited to the TCR, where they co-localized with activated ZAP-70 and then migrated to the periphery where they accumulated at an actin-rich circumferential ring.

In conclusion, advances in confocal microscopy and imaging techniques, such as FRET and time-resolved imaging of fluorescent chimeras, enabled our study of actin polymerization and reorganization at the TCR. This process plays a major role in immunological synapse formation and amplification of the immune response.

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■ CELL BIOLOGY

Chromatin Epigenetics: Nucleosome-binding Proteins Modulate the Levels of Histone Posttranslational Modifications in Chromatin

Lim JH, West KL, Rubinstein Y, Bergel M, Postnikov YV, and Bustin M. Chromosomal protein HMGN1 enhances the acetylation of lysine 14 in histone H3. *EMBO J* 24: 3038–48, 2005.

Lim JH, Catez F, Birger Y, West KL, Prymakowska-Bosak M, Postnikov YV, and Bustin M. Chromosomal protein HMGN1 modulates histone H3 phosphorylation. *Mol Cell* 15: 573–84, 2004.

Posttranslational modifications in the tails of the nucleosomal histones are important epigenetic markers and serve as potential targets for cancer therapy. In living cells, the levels of histone modifications are not fixed. They are in a continuous state of flux and reflect the equilibrium reached between the activities of enzymes that continuously modify and those that continuously demodify specific histone residues. For example, the equilibrium between the opposing activities of histone acetylases (HATs) and deacetylases (HDACs) or kinases and phosphatases determines the levels of acetylation and phosphorylation in chromatin. Recruitment of histone-modifying or -demodifying enzyme complexes to specific sites is part of the mechanism that regulates gene expression.

We explored the possibility that chromatin-binding structural proteins, such as HMGNs, which are devoid of enzymatic activity, are part of the mechanism that regulates the levels of chromatin modifications. HMGNs are a family of proteins that bind to nucleosomes without specificity for the underlying DNA sequence and induce structural and functional changes in chromatin. We reasoned that the binding of these proteins to nucleosomes may induce local or global changes in chromatin and shift the equilibrium between the activities of histone-modifying and -demodifying enzymes. To test this hypothesis, we used specific antibodies to examine the levels of several modifications in the tail of histone H3, isolated from either wild-type or from *Hmgn1*$^{-/-}$ mouse embryonic fibroblasts (MEFs). We found that loss of HMGN1 protein elevated the levels of phosphorylation at serine 10 (H3S10p) and serine 28 (H3S28p) but decreased the levels of acetylation at lysine 14 (H3K14ac). Reexpression of the HMGN1 in the *Hmgn1*$^{-/-}$ cells, by induction of stably integrated vectors, elevated the levels of H3K14ac and reduced the levels of H3S10p, proof that the levels of these modifications are indeed regulated by HMGN1. Significantly, reexpression of a mutant HMGN1 that does not bind to chromatin did not alter the levels of these modifications, an indication that HMGN1 modulates histone modifications by binding to chromatin.

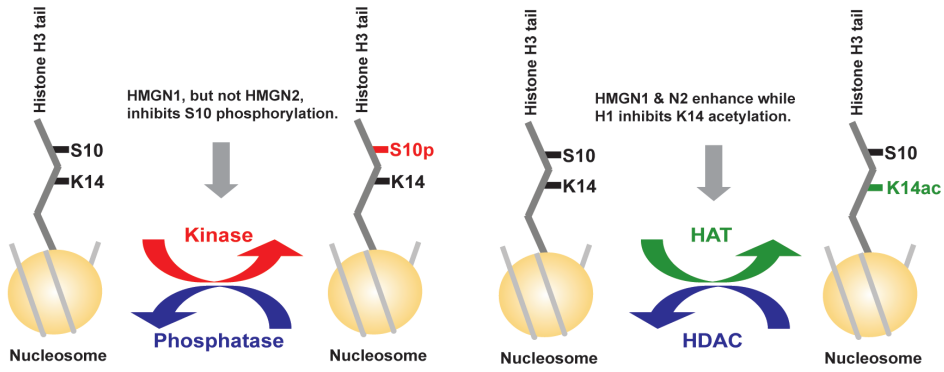

Figure 1. Model illustrating the effects of the structural chromatin-binding proteins H1, HMGN1, and HMGN2 on the levels of specific histone modifications. These proteins affect the levels of histone modifications by altering the dynamic equilibrium between the enzymes that continuously add or remove chemical tags, such as acetyl or phosphate groups, to histone tails. N2, HMGN2; S10, serine 10; K14, lysine 14; S10p, phosphorylated S10; H1, histone 1; K14ac, acetylated K14; HAT, histone acetylase; HDAC, histone deacetylase.

Further *in vitro* studies and analysis of cells treated with HDAC inhibitors revealed that HMGN1 elevates the levels of H3K14ac by enhancing the activity of a specific HAT, rather than by reducing the demodifying activity of an HDAC. Similar studies on the mechanisms whereby HMGN1 reduces the levels of H3S10p indicate that the binding of the protein to nucleosomes hinders the ability of specific kinases to access and modify their targets. The *in vivo* studies are fully supported by *in vitro* analyses indicating that HMGN1 affects these H3 modifications only in the context of chromatin. The modification of purified H3 protein is not affected by HMGN1. Thus, HMGN1 can either enhance or reduce the level of a specific modification in chromatin.

Interestingly, a close homolog of HMGN1, named HMGN2, enhanced the acetylation of H3K14 more efficiently than HMGN1 but did not inhibit the phosphorylation of H3S10, suggesting HMGN variant–specific effects on histone modification. Current analysis of a series of mutants in which distinct domains of HMGN1 were swapped with domains of HMGN2 indicate that distinct domains of the proteins are involved in enhancement of acetylation and reduction of phosphorylation, further proof for HMGN-specific effects on histone modifications.

We have previously demonstrated that the linker histone H1 inhibits the acetylation of H3K14 (Herrera JE et al. *Mol Cell Biol* 20: 523–9, 2000). Taken together, these studies establish that chromatin-binding structural proteins modulate the levels of chromatin modifications, most likely by altering the ability of chromatin modifiers to access and modify their targets. HMGNs and H1 may affect the accessibility of sites in chromatin either by inducing global changes in the "compaction" of the chromatin fiber, or by inducing steric changes at the local levels of the nucleosome. Our studies demonstrate that structural proteins alter the equilibrium generated by the activities of the enzymes that determine the levels of chromatin modifications, and point to an additional mechanism that regulates these epigenetic markers (Figure 1).

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VIROLOGY

We Keep Learning from Retroviruses

Smulevitch S, Michalowski D, Zolotukhin AS, Schneider R, Bear J, Roth P, Pavlakis GN, and Felber BK. Structural and functional analysis of the RNA transport element, a member of an extensive family present in the mouse genome. *J Virol* 79: 2356–65, 2005.

Retroviruses gave us oncogenes, and their study helped in the elucidation of molecular mechanisms of carcinogenesis. However, this is not all. Retroviruses and retroelements keep us busy in the discovery and the refining of our understanding of basic mechanisms mediating gene expression. Posttranscriptional regulation is a critical step in mRNA metabolism that controls the levels of gene expression of both viral and cellular genes. The detailed analysis of the fundamental cellular processes that guide the complex assembly of mRNA and proteins and their transport from the nucleus to the cytoplasm is essential for understanding the regulation of gene expression.

Research over approximately the past 20 years has revealed that many retroviruses depend on elaborate mechanisms for nucleocytoplasmic export of their unspliced, full-length RNA. These transcripts encode the Gag-pol polyprotein and, in addition, serve as genomic RNA to be packaged into progeny virions in the cytoplasm. Studies of the molecular

biology of HIV-1 have been instrumental for major discoveries in the field of mRNA metabolism and macromolecule transport. In HIV-1 and other lentiviruses, this process depends on the Rev protein, which is essential for the production of structural proteins and infectious virions (Figure 1). Rev promotes export and expression of *gag/pol* and *env* mRNAs by binding to the *cis*-acting RNA recognition signal, the Rev-responsive element (RRE). A similar mechanism is essential for human T-cell leukemia virus (HTLV) and human endogenous retrovirus (HERV-K) expression. Rev and its functional homologs, as well as many cellular proteins, share a leucine-rich nuclear export signal, which is recognized by the cellular export receptor CRM1, thereby linking the mRNP (mRNA-protein) cargo to the nuclear pore complex. The discovery of the Rev export pathway paved the way to understanding the trafficking of cellular proteins such as MDM2, MAPKK, PKI, and others.

Expression of simian type D retrovirus (SRV/MPMV) depends on the cellular *trans*-acting factor TAP/NXF1, which binds to the *cis*-acting constitutive transport element (CTE) (Figure 1). The extended stem-loop structure of CTE is conserved among all type D species. A CTE-related element was found in a subgroup of rodent intracisternal A particle retroelements (IAPs), and more than 100 CTE-related elements are present in the mouse genome. The cellular NXF1 is not only the export receptor of CTE-containing RNA, but most importantly, it is the key export factor for cellular mRNAs, a function that is conserved among eukaryotes.

We discovered another potent RNA transport element (RTE, Figure 1), linked to a "fossilized" mouse IAP (Nappi F et al. *J Virol* 75: 4558–69, 2001). RTE is functionally similar, but structurally unrelated, to CTE and also functions in many cell types of different species, indicating that its export factor(s) are widely expressed and evolutionarily conserved. RTE does not bind the export factor NXF1 and mediates mRNA export via interactions with other still unknown cellular factor(s). Similar to CTE, RTE

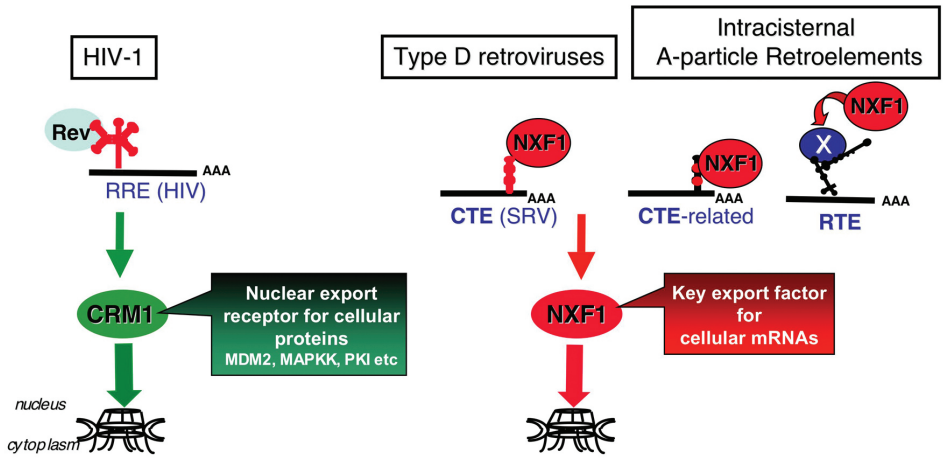

Figure 1. Distinct export pathways from the nucleus. CRM1 and NXF1 represent two key export pathways from the nucleus. Studies of retroviruses and retroelements have been critical for their discovery. CRM1 is essential for the Rev-mediated HIV mRNA export and expression. NXF1 is the molecular link between the constitutive transport element (CTE)– and RNA transport element (RTE)–containing mRNAs and the nuclear pore complex. The key factors mediating mRNA export of retrovirus and retroelement mRNA export also promote transport of cellular mRNAs and proteins. Rev, HIV-1 protein essential for the production of structural proteins and infectious virions; RRE, Rev-responsive element; SRV, simian type D retrovirus.

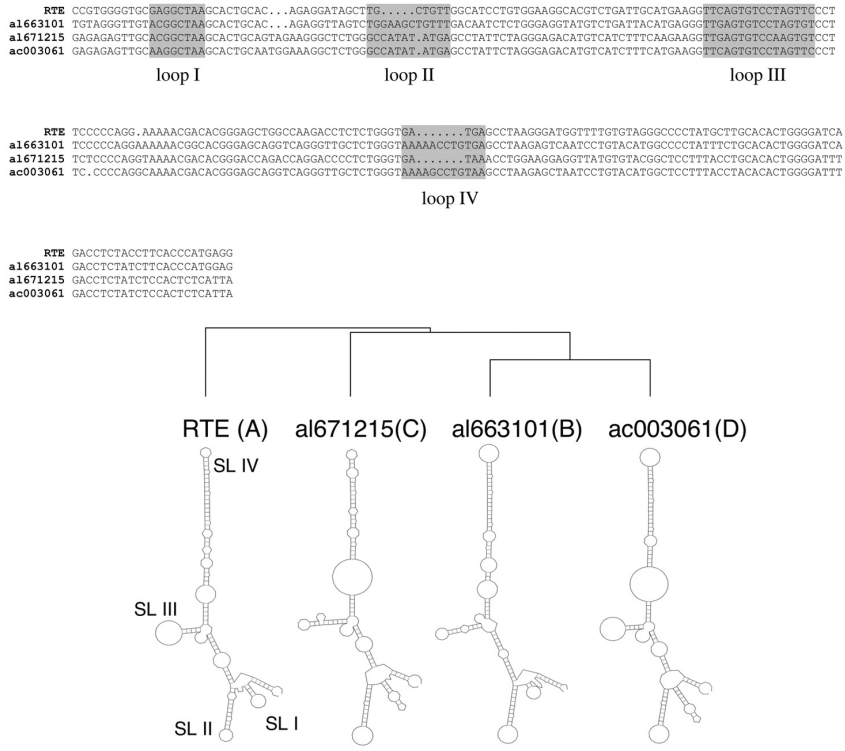

Figure 2. Family of RTE-related elements in the mouse genome. The 226-nucleotide (nt)–spanning RTE was used to identify related elements in the mouse genome. Based on structure and sequence, these elements form four groups. The top panel shows the alignment of the RTE and a representative member of each group of RTE-related elements found in GenBank entries al663101 (Group B), al671215 (Group C), and ac003061 (Group D). The shaded areas indicate the loops defined for RTE. Phylogenetic tree and comparison of the identified RTE structure and the predicted secondary structures for the RTE-related elements are shown (bottom panel). SL I, II, III, and IV indicate the identified stem-loop (SL) structures with the RTE.

depends on a conserved cellular transport mechanism, which makes this mRNA export element a valuable tool for further understanding the mRNA nucleocytoplasmic transport.

Here, using computer prediction supported by experimental RNA structure analysis, we found that RTE folds into a novel, extended RNA secondary structure (Figure 2) (Smulevitch S et al. *J Virol* 79: 2356–65, 2005). Detailed mutational analysis revealed that the minimal RTE contains four internal stem-loops that are indispensable for function in mammalian cells. Therefore, the RTE depends on a complex secondary structure, which is important for the interaction with cellular export factor(s). Sequence similarity analyses revealed that in addition to more than 100 identical RTEs, there are more than 3,000 RTE-related elements in the mouse genome, which share at least 70% sequence identity and which can be found on all the chromosomes. The predicted key structural features of RTE are preserved among these related elements, consistent with their functional importance. Based on their sequence and structure, these elements form four subgroups (Figure 2, structures A through D).

Our research on posttranscriptional elements has further given new insights into the biology of retroelements and their potential effect to alter cellular gene expression. We found that IAP retroelements are more complex than previously thought and that they fall into at least two subfamilies depending on the presence of either the CTE- or the RTE-related RNA export elements. Using an active IAP, we recently found that removal of its RTE leads to abolishment of retrotransposition. This experiment showed for the first time that posttranscriptional control is essential not only for retroviruses but also for long terminal repeat retroelements. Our findings suggest that active RNA export elements are inserted into genes via retrotransposition and can thereby affect the posttranscriptional regulation of cellular gene expression. The presence of the many RTEs in the genome provides us with important new information about posttranscriptional regulation, genome organization, genome evolution, and the potential of IAPs to affect cellular gene expression, which may lead to carcinogenesis.

This experiment showed for the first time that posttranscriptional control is essential not only for retroviruses but also for long terminal repeat retroelements.

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