

- Escher, A., E. Pietet, F. Waridel, R. Iggo and P. Monnier (2009). "p53 Mutation in histologically normal mucosa of the aero-digestive tract is not a marker of increased risk for second primary carcinoma in head and neck cancer patients." **Eur Arch Otorhinolaryngol** 266: 547-51 Head and neck cancer patients are at high risk for developing second primary tumors. This is known as field cancerization of the aero-digestive tract. In a previous study, we showed that patients with multiple primary tumors were more likely to have p53 mutations in histologically normal mucosae than patients presenting with an isolated tumor. Based on this observation, we postulated that p53 mutations in normal tissue samples of patients bearing a single primary tumor could have a clinical value as a biomarker for the risk of developing second primary tumors. Thirty-five patients presenting with a single primary tumor were followed-up for a median of 51 months (range 1 month to 10.9 years) after biopsies of histologically normal squamous cell mucosa had been analyzed for p53 mutations with a yeast functional assay at the time of the primary tumor. During this follow-up, recurrences and non-sterilization of the primary tumor, occurrence of lymph node metastases, and of second primary tumors were evaluated. Sixteen (45.7%) patients were found to have p53 mutations in their normal squamous cell mucosa, and 19 (54.3%) patients showed no mutation. No relationship was found between p53 mutations and the occurrence of evaluated events during follow-up. Notably, the rate of second primary tumors was not associated with p53 mutations in the normal squamous mucosa. The correlation between p53 mutations in histologically normal mucosae and the incidence of second primary tumors is generally low. The benefit of analyzing p53 mutations in samples of normal squamous cell mucosa in every patient with a primary tumor of the head and neck is doubtful.
- Farmer, P., H. Bonnefoi, P. Anderle, D. Cameron, P. Wirapati, V. Becette, S. Andre, M. Piccart, M. Campone, E. Brain, G. Macgrogan, T. Petit, J. Jassem, F. Bibeau, E. Blot, J. Bogaerts, M. Aguet, J. Bergh, R. Iggo and M. Delorenzi (2009). "A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer." **Nat Med** 15: 68-74 To better understand the relationship between tumor-host interactions and the efficacy of chemotherapy, we have developed an analytical approach to quantify several biological processes observed in gene expression data sets. We tested the approach on tumor biopsies from individuals with estrogen receptor-negative breast cancer treated with chemotherapy. We report that increased stromal gene expression predicts resistance to preoperative chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC) in subjects in the EORTC 10994/BIG 00-01 trial. The predictive value of the stromal signature was successfully validated in two independent cohorts of subjects who received chemotherapy but not in an untreated control group, indicating that the signature is predictive rather than prognostic. The genes in the signature are expressed in reactive stroma, according to reanalysis of data from microdissected breast tumor samples. These findings identify a previously undescribed resistance mechanism to FEC treatment and suggest that antistromal agents may offer new ways to overcome resistance to chemotherapy.
- Bazan-Peregrino, M., R. C. Carlisle, R. Hernandez-Alcoceba, R. Iggo, K. Homicsko, K. D. Fisher, G. Hallden, V. Mautner, Y. Shen and L. W. Seymour (2008). "Comparison of molecular strategies for breast cancer virotherapy using oncolytic adenovirus." **Hum Gene Ther** 19: 873-86 Oncolytic viruses are regulated by the tumor phenotype to replicate and lyse cancer cells selectively. To identify optimal strategies for breast cancer we compared five adenoviruses with distinct regulatory mechanisms: Ad-dl922-947 (targets G1-S checkpoint); Ad-Onyx-015 and Ad-Onyx-017 (target p53/mRNA export); Ad-vKH1 (targets Wnt pathway), and AdEHE2F (targets estrogen receptor/G1-S checkpoint/hypoxic signaling). The quantity of virus required to kill 50% of breast cancer cells after 6 days (EC(50), plaque-forming units per cell) was measured. The most potent virus was Ad-dl922-947 (EC(50), 0.01-5.4 in SkBr3, MDA-231, MDA-468, MCF7, and ZR75.1 cells), followed by wild-type (Ad-WT; EC(50), 0.3-5.5) and AdEHE2F (EC(50), 1.4-3.9). Ad-vKH1 (EC(50), 7.2-72.1), Ad-Onyx-017 (EC(50), 8.4-167), and Ad-Onyx-015 (EC(50), 17.7-377) showed less activity. Most viruses showed limited cytotoxicity in normal human cells, including breast epithelium MCF10A (EC(50), >722) and fibroblasts (EC(50), >192) and only moderate cytotoxicity in normal microvascular endothelial cells (HMVECs; EC(50), 42.8-149), except Ad-dl922-947, which was active in HMVECs (EC(50), 1.6). After

injection into MDA-231 xenografts, Ad-WT, AdEHE2F, and Ad-dl922-947 showed replication, assessed by hexon staining and quantitative polymerase chain reaction measurement of viral DNA, and significantly inhibited tumor growth, leading to extended survival. After intravenous injection Ad-dl922-947 showed DNA replication (233% of the injected dose was measured in liver after 3 days) whereas AdEHE2F did not. Overall, AdEHE2F showed the best combination of low toxicity in normal cells and high activity in breast cancer in vitro and in vivo, suggesting that molecular targeting using estrogen response elements, hypoxia response elements, and a dysregulated G1-S checkpoint is a promising strategy for virotherapy of breast cancer.

Funston, G. M., S. E. Kallioinen, P. de Felipe, M. D. Ryan and R. D. Iggo (2008). "Expression of heterologous genes in oncolytic adenoviruses using picornaviral 2A sequences that trigger ribosome skipping." **J Gen Virol** 89: 389-96

Insertion of picornaviral 2A sequences into mRNAs causes ribosomes to skip formation of a peptide bond at the junction of the 2A and downstream sequences, leading to the production of two proteins from a single open reading frame. Adenoviral protein IX is a minor capsid protein that has been used to display foreign peptides on the surface of the capsid. We have used 2A sequences from the foot-and-mouth disease virus (FMDV) and porcine teschovirus 1 (PTV-1) to express protein IX (pIX) and green fluorescent protein (GFP) from pIX-2A-GFP fusion genes in an oncolytic virus derived from human adenovirus 5. GFP was efficiently expressed by constructs containing either 2A sequence. Peptide bond skipping was more efficient with the 58 aa FMDV sequence than with the 22 aa PTV-1 2A sequence, but the virus with the FMDV 2A sequence showed a reduction in plaque size, cytopathic effect, viral burst size and capsid stability. We conclude that ribosome skipping induced by 2A sequences is an effective strategy to express heterologous genes in adenoviruses; however, careful selection or optimization of the 2A sequence may be required if protein IX is used as the fusion partner.

Lukashev, A. N., O. E. Ivanova, T. P. Eremeeva and R. D. Iggo (2008). "Evidence of frequent recombination among human adenoviruses." **J Gen Virol** 89: 380-8

Genome stability is a prerequisite for the production and use of adenoviruses for therapy of genetic diseases and cancer. To test the premise that the adenoviral genome is stable, the phylogenetic relationships of 16 adenovirus C (AdC) field isolates were studied in four genome regions: hexon, fiber, polymerase and E1A. The phylogenetic relationships in the fiber gene concurred with those in the hexon region. In contrast, the non-structural regions had marks of frequent recombination, to the point that an isolate of one serotype could contain non-structural proteins that were identical to the genes from a different serotype. Our results suggest that recombination among circulating adenoviruses is very frequent and plays an important role in shaping the phylogenetic relationships of adenovirus genomes. Analysis of the available complete genome sequences of AdB, AdC and AdD species showed that recombination shuffles genome fragments within a species, but not between species. One of the AdC field isolates possessed the fiber gene of AdC type 6, but a hexon gene that was distinct from all AdC serotypes. This strain could not be typed unambiguously in a neutralization test and might represent a novel serotype of AdC. Comparison of the right end (nt 18838-33452) of this isolate with that of the ATCC Ad6 strain showed clear evidence of multiple recombination events.

Peerlinck, I., S. Amini-Nik, R. K. Phillips, R. Iggo, N. R. Lemoine, S. Tejpar and G. Vassaux (2008). "Therapeutic potential of replication-selective oncolytic adenoviruses on cells from familial and sporadic desmoid tumors." **Clin Cancer Res** 14: 6187-92

PURPOSE: Constitutive activation of the Wnt signaling pathway is a hallmark of many cancers and has been associated with familial and sporadic desmoid tumors. The aim of the present study is to assess the therapeutic potential of oncolytic adenoviruses selectively replicating in cells in which the Wnt signaling pathway is active on primary cells from desmoid tumors. **EXPERIMENTAL DESIGN:** Primary cells extracted from familial (n = 3) or sporadic (n = 3) desmoid tumors were cultured short term. Cancer cell survival and viral replication were measured in vitro upon infection with two different oncolytic adenoviruses targeting a constitutive activation of the Wnt signaling pathway. Adenoviral infectivity was also assessed. **RESULTS:** Although cells extracted from one sporadic desmoid tumor responded very well to the

oncolytic action of the adenoviruses (<20% of viable cells upon infection at a multiplicity of infection of 10), cells from two tumor samples were totally resistant to the viral action. Cells from the remaining samples showed intermediate sensitivity to the oncolytic viruses. These effects were correlated to the level of infectivity of the cells. Finally, in responder cells, evidences of viral replication was observed.

CONCLUSIONS: Our experimental data suggest that the response of desmoid tumor cells to oncolytic adenovirus is neither correlated to the type of mutation activating the Wnt signaling pathway nor to the familial or sporadic nature of the tumor. In addition, they highlight the variability of infectivity of individual tumors and predict a great variability in the response to oncolytic adenoviruses.

Seymour, L. W., M. Bazan-Peregrino, R. Carlisle, R. Hernandez-Alcoceba, R. Iggo, K. Homicsko, G. Hallden, V. Mautner, J. Shen and K. Fisher (2008). "Comparison of Molecular Strategies for Breast Cancer Virotherapy using Oncolytic Adenovirus." **Hum Gene Ther** Oncolytic viruses are regulated by the tumour phenotype to replicate and lyse cancer cells selectively. To identify optimal strategies for breast cancer we compared five adenoviruses with distinct regulatory mechanisms: Ad-dl922-947 (targets G1-S checkpoint); Ad-Onyx-015 and Ad-Onyx-017 (target p53/mRNA export); Ad-vKH1 (targets Wnt pathway) and AdEHE2F (targets estrogen receptor/G1-S checkpoint/hypoxic signalling). The quantity of virus required to kill 50% of breast cancer cells after 6 days (EC50, PFU/cell) was measured. The most potent virus was Ad-dl922-947 (EC50 0.01-5.4 in SkBr3, MDA-231, MDA-468, MCF7, ZR75.1), followed by wild type (Ad-WT; EC50 0.3-5.5) and AdEHE2F (EC50 1.4-3.9). Ad-vKH1 (EC50 7.2-72.1), Ad-Onyx-017 (EC50 8.4-167) and Ad-Onyx-015 (EC50 17.7-377) showed less activity. Most viruses showed very limited cytotoxicity in normal human cells, including breast epithelium MCF10A (EC50 >722) and fibroblasts (EC50 >192) and only moderate cytotoxicity in normal microvascular endothelial cells (HMVEC, EC50 42.8-149), except Ad-dl922-947 which was active in HMVEC (EC50 1.6). After injection into MDA-231 xenografts, Ad-WT, AdEHE2F and Ad-dl922-947 showed replication, assessed by hexon staining and quantitative PCR measurement of viral DNA, and significantly inhibited tumour growth leading to extended survival. Following intravenous injection Ad-dl922-947 showed DNA replication (233% of the injected dose was measured in liver after 3 days) while AdEHE2F did not. Overall, AdEHE2F showed the best combination of low toxicity in normal cells and high activity in breast cancer in vitro and in vivo, suggesting that molecular targeting using estrogen response elements, hypoxia response elements and dysregulated G1-S checkpoint provides a good strategy for virotherapy of breast cancer.

Duss, S., S. Andre, A. L. Nicolaz, M. Fiche, H. Bonnefoi, C. Brisken and R. D. Iggo (2007). "An oestrogen-dependent model of breast cancer created by transformation of normal human mammary epithelial cells." **Breast Cancer Res** 9: R38 **INTRODUCTION:** About 70% of breast cancers express oestrogen receptor alpha (ESR1/ERalpha) and are oestrogen-dependent for growth. In contrast with the highly proliferative nature of ERalpha-positive tumour cells, ERalpha-positive cells in normal breast tissue rarely proliferate. Because ERalpha expression is rapidly lost when normal human mammary epithelial cells (HMECs) are grown in vitro, breast cancer models derived from HMECs are ERalpha-negative. Currently only tumour cell lines are available to model ERalpha-positive disease. To create an ERalpha-positive breast cancer model, we have forced normal HMECs derived from reduction mammoplasty tissue to express ERalpha in combination with other relevant breast cancer genes. **METHODS:** Candidate genes were selected based on breast cancer microarray data and cloned into lentiviral vectors. Primary HMECs prepared from reduction mammoplasty tissue were infected with lentiviral particles. Infected HMECs were characterised by Western blotting, immunofluorescence microscopy, microarray analysis, growth curves, karyotyping and SNP chip analysis. The tumorigenicity of the modified HMECs was tested after orthotopic injection into the inguinal mammary glands of NOD/SCID mice. Cells were marked with a fluorescent protein to allow visualisation in the fat pad. The growth of the graft was analysed by fluorescence microscopy of the mammary glands and pathological analysis of stained tissue sections. Oestrogen dependence of tumour growth was assessed by treatment with the oestrogen antagonist fulvestrant. **RESULTS:** Microarray analysis of ERalpha-

positive tumours reveals that they commonly overexpress the Polycomb-group gene BMI1. Lentiviral transduction with ERalpha, BMI1, TERT and MYC allows primary HMECs to be expanded in vitro in an oestrogen-dependent manner. Orthotopic xenografting of these cells into the mammary glands of NOD/SCID mice results in the formation of ERalpha-positive tumours that metastasise to multiple organs. The cells remain wild type for TP53, diploid and genetically stable. In vivo tumour growth and in vitro proliferation of cells explanted from tumours are dependent on oestrogen. CONCLUSION: We have created a genetically defined model of ERalpha-positive human breast cancer based on normal HMECs that has the potential to model human oestrogen-dependent breast cancer in a mouse and enables the study of mechanisms involved in tumorigenesis and metastasis.

- Merron, A., I. Peerlinck, P. Martin-Duque, J. Burnet, M. Quintanilla, S. Mather, M. Hingorani, K. Harrington, R. Iggo and G. Vassaux (2007). "SPECT/CT imaging of oncolytic adenovirus propagation in tumours in vivo using the Na/I symporter as a reporter gene." **Gene Ther** 14: 1731-8 Oncolytic adenoviruses have shown some promise in cancer gene therapy. However, their efficacy in clinical trials is often limited, and additional therapeutic interventions have been proposed to increase their efficacies. In this context, molecular imaging of viral spread in tumours could provide unique information to rationalize the timing of these combinations. Here, we use the human sodium iodide symporter (hNIS) as a reporter gene in wild-type and replication-selective adenoviruses. By design, hNIS cDNA is positioned in the E3 region in a wild-type adenovirus type 5 (AdIP1) and in an adenovirus in which a promoter from the human telomerase gene (RNA component) drives E1 expression (AdAM6). Viruses show functional hNIS expression and replication in vitro and kinetics of spread of the different viruses in tumour xenografts are visualized in vivo using a small animal nano-SPECT/CT camera. The time required to reach maximal spread is 48 h for AdIP1 and 72 h for AdAM6 suggesting that genetic engineering of adenoviruses can affect their kinetics of spread in tumours. Considering that this methodology is potentially clinically applicable, we conclude that hNIS-mediated imaging of viral spread in tumours may be an important tool for combined anticancer therapies involving replicating adenoviruses
- Takahashi, M., H. Shimodaira, C. Andreutti-Zaugg, R. Iggo, R. D. Kolodner and C. Ishioka (2007). "Functional analysis of human MLH1 variants using yeast and in vitro mismatch repair assays." **Cancer Res** 67: 4595-604 The functional characterization of nonsynonymous single nucleotide polymorphisms in human mismatch repair (MMR) genes has been critical to evaluate their pathogenicity for hereditary nonpolyposis colorectal cancer. We previously established an assay for detecting loss-of-function mutations in the MLH1 gene using a dominant mutator effect of human MLH1 expressed in *Saccharomyces cerevisiae*. The purpose of this study is to extend the functional analyses of nonsynonymous single nucleotide polymorphisms in the MLH1 gene both in quality and in quantity, and integrate the results to evaluate the variants for pathogenic significance. The 101 MLH1 variants, which covered most of the reported MLH1 nonsynonymous single nucleotide polymorphisms and consisted of one 3-bp deletion, 1 nonsense and 99 missense variants, were examined for the dominant mutator effect by three yeast assays and for the ability of the variant to repair a heteroduplex DNA with mismatch bases by in vitro MMR assay. There was diversity in the dominant mutator effects and the in vitro MMR activities among the variants. The majority of functionally inactive variants were located around the putative ATP-binding pocket of the NH(2)-terminal domain or the whole region of the COOH-terminal domain. Integrated functional evaluations contribute to a better prediction of the cancer risk in individuals or families carrying MLH1 variants and provide insights into the function-structure relationships in MLH1.
- Fuerer, C., K. Homicsko, A. N. Lukashev, A. L. Pittet and R. D. Iggo (2006). "Fusion of the BCL9 HD2 domain to E1A increases the cytopathic effect of an oncolytic adenovirus that targets colon cancer cells." **BMC Cancer** 6: 236 BACKGROUND: The Wnt signaling pathway is activated by mutations in the APC and beta-catenin genes in many types of human cancer. beta-catenin is stabilized by these mutations and activates transcription in part by acting as a bridge between Tcf/LEF proteins and the HD2 domain of the BCL9 coactivator. We have previously described oncolytic adenoviruses with binding sites for Tcf/LEF transcription factors inserted into the

early viral promoters. These viruses replicate selectively in cells with activation of the Wnt pathway. To increase the activity of these viruses we have fused the viral transactivator E1A to the BCL9 HD2 domain. METHODS: Luciferase assays, co-immunoprecipitation and Western blotting, immunofluorescent cell staining and cytopathic effect assays were used to characterize the E1A-HD2 fusion protein and virus in vitro. Growth curves of subcutaneous SW620 colon cancer xenografts were used to characterize the virus in vivo. RESULTS: The E1A-HD2 fusion protein binds to beta-catenin in vivo and activates a Tcf-regulated luciferase reporter better than wild-type E1A in cells with activated Wnt signaling. Expression of the E1A-HD2 protein promotes nuclear import of beta-catenin, mediated by the strong nuclear localization signal in E1A. Tcf-regulated viruses expressing the fusion protein show increased expression of viral proteins and a five-fold increase in cytopathic effect (CPE) in colorectal cancer cell lines. There was no change in viral protein expression or CPE in HeLa cells, indicating that E1A-HD2 viruses retain selectivity for cells with activation of the Wnt signaling pathway. Despite increasing the cytopathic effect of the virus in vitro, fusion of the HD2 domain to E1A did not increase the burst size of the virus in vitro or the anti-tumor effect of the virus in an SW620 xenograft model in vivo. CONCLUSION: Despite an increase in the nuclear pool of beta-catenin, the effects on viral activity in colon cancer cells were small, suggesting that factors acting downstream of beta-catenin are limiting for viral replication and toxicity in these cells. The approach of fusing E1A to a protein domain implicated in oncogenic signaling could be used to selectively increase the activity of oncolytic viruses targeting several other pathways defective in cancer.

Malerba, M., D. Nikolova, J. Cornelis and R. Iggo (2006). "Targeting of autonomous parvoviruses to colon cancer by insertion of Tcf sites in the P4 promoter." **Cancer Gene Ther** 13: 273-80 The Wnt signaling pathway is activated by mutations in the adenomatous polyposis coli (APC) or beta-catenin genes in most colon cancers, leading to the transactivation of promoters containing binding sites for the Tcf/LEF family of transcription factors. We have previously shown that it is possible to confer colon cancer specificity on autonomous parvoviruses by inserting Tcf sites into the viral P4 promoter. The mutant Tcf promoters were responsive to activation of the Wnt pathway but the viruses replicated poorly. We show here that reduction of the number of Tcf sites from four to two leads to an increase in the efficiency of replication and toxicity of the viruses in Co115 colon cancer cells, with only a small reduction in selectivity for cells with an active Wnt signaling pathway. Despite this improvement, virus production by most colon cancer cells remained low. Analysis of parental pH1 virus infection of SW480 colon cancer cells showed that the nonstructural and capsid proteins were expressed, but single stranded DNA and progeny virus were not produced. This defect reflects the dependence of autonomous parvoviruses on host functions for many steps in their replication cycle and represents a major limitation to the use of selectively replicating parvoviruses for colon cancer therapy.

Farmer, P., H. Bonnefoi, V. Becette, M. Tubiana-Hulin, P. Fumoleau, D. Larsimont, G. Macgrogan, J. Bergh, D. Cameron, D. Goldstein, S. Duss, A. L. Nicoulaz, C. Briskin, M. Fiche, M. Delorenzi and R. Iggo (2005). "Identification of molecular apocrine breast tumours by microarray analysis." **Oncogene** 24: 4660-71 Previous microarray studies on breast cancer identified multiple tumour classes, of which the most prominent, named luminal and basal, differ in expression of the oestrogen receptor alpha gene (ER). We report here the identification of a group of breast tumours with increased androgen signalling and a 'molecular apocrine' gene expression profile. Tumour samples from 49 patients with large operable or locally advanced breast cancers were tested on Affymetrix U133A gene expression microarrays. Principal components analysis and hierarchical clustering split the tumours into three groups: basal, luminal and a group we call molecular apocrine. All of the molecular apocrine tumours have strong apocrine features on histological examination (P=0.0002). The molecular apocrine group is androgen receptor (AR) positive and contains all of the ER-negative tumours outside the basal group. Kolmogorov-Smirnov testing indicates that oestrogen signalling is most active in the luminal group, and androgen signalling is most active in the molecular apocrine group. ERBB2 amplification is commoner in the molecular apocrine than the other groups. Genes that best split the three groups were identified by Wilcoxon test. Correlation of the average expression profile of

these genes in our data with the expression profile of individual tumours in four published breast cancer studies suggest that molecular apocrine tumours represent 8-14% of tumours in these studies. Our data show that it is possible with microarray data to divide mammary tumour cells into three groups based on steroid receptor activity: luminal (ER+ AR+), basal (ER- AR-) and molecular apocrine (ER- AR+).

- Homicksko, K., A. Lukashev and R. D. Iggo (2005). "RAD001 (everolimus) improves the efficacy of replicating adenoviruses that target colon cancer." **Cancer Res** 65: 6882-90
- Selectively replicating adenoviruses have the potential to cure cancer but have shown little efficacy in clinical trials. We have tested the ability of the mTOR kinase inhibitor RAD001 (everolimus) to enhance the response of xenografts to an oncolytic adenovirus. The virus has Tcf sites inserted in the early viral promoters and replicates selectively in cells with activation of the Wnt signaling pathway. To enhance tumor cell infection, an integrin targeting peptide (CDCRGDCFC) was inserted into the fiber gene of the virus. RAD001 combines three useful properties: it inhibits tumor cell growth directly, blocks angiogenesis, and suppresses the immune response. RAD001 does not block viral protein expression, DNA replication, or cytopathic effect in tumor cells in vitro. After 6 weeks of daily RAD001 treatment, ongoing viral DNA replication could be detected in tumor xenografts, showing that RAD001 does not inhibit virus replication in vivo. I.v. injection of virus alone produced a small delay in xenograft growth, whereas combination therapy substantially prolonged the survival of the mice. We suggest that collapsing the tumor vasculature after the initial infection traps the virus and facilitates local spread within the tumor. Unlike conventional drugs, which require continued access to the tumor through the vascular system, oncolytic viruses are in principle less sensitive to late reductions in perfusion because they are produced locally within the tumor.
- Lukashev, A. N., C. Fuerer, M. J. Chen, P. Searle and R. Iggo (2005). "Late expression of nitroreductase in an oncolytic adenovirus sensitizes colon cancer cells to the prodrug CB1954." **Hum Gene Ther** 16: 1473-83
- We have constructed an oncolytic adenovirus expressing the Escherichia coli nitroreductase gene *nfsB* from an internal ribosome entry site (IRES) in the adenovirus L5 major late transcript. The virus (Tcf-NTR) has Tcf transcription factor-binding sites in the E1A, E1B, and E4 promoters, which restrict viral replication to cells that have activation of the Wnt signaling pathway. This virus was compared with an E1B-55K-deleted virus expressing nitroreductase (NTR) from a cytomegalovirus (CMV) promoter in the E1B-55K region [CRAAd-NTR(PS1217H6)]. Both viruses express NTR in colorectal cancer cell lines and show increased cytopathic effect in the presence of the prodrug CB1954. Unlike the Tcf-NTR virus, the CMV-NTR virus expresses NTR in human lung fibroblasts and sensitizes these normal cells to CB1954. The in vivo activity of the viruses was tested in SW620 xenografts in nude mice by intravenous injection of 1,011 particles of virus followed 1 week later by intraperitoneal injections of CB1954. The CMV-NTR virus produced minimal effects in this model. The median time to form 1,000-mm³ tumors in mice treated with the Tcf-NTR virus plus CB1954 was increased from 14 to 26 days (p=0.003), but this was due mainly to the direct oncolytic effect of the virus. Combination therapy with 3 x 10¹¹ particles of Tcf-NTR virus (given intravenously) and the mammalian target of rapamycin (mTOR) inhibitor RAD001 (everolimus) (given orally) significantly improved survival (median, >50 days), and addition of CB1954 to this regimen further delayed tumor growth. These results show that the Tcf-NTR virus is more tumor selective and active than the CMV-NTR virus. At the level of transduction that can be achieved currently with oncolytic viruses given intravenously, drugs such as RAD001, which do not require activation by the virus, produce greater increases in efficacy than prodrugs such as CB1954.
- Fuerer, C. and R. Iggo (2004). "5-Fluorocytosine increases the toxicity of Wnt-targeting replicating adenoviruses that express cytosine deaminase as a late gene." **Gene Ther** 11: 142-51
- Clinical studies with oncolytic adenoviruses have shown that existing viruses are safe but lack efficacy. To selectively increase the toxicity of oncolytic adenoviruses targeting colon tumours, we have inserted the yeast cytosine deaminase gene (*yCD*) after the fibre gene in the major late transcript. *yCD* was expressed using either an internal ribosome entry site (IRES) or by alternative splicing of a new exon analogous to the Ad41 long fibre exon. The IRES-CD virus gave higher *yCD* expression on Western blots. Both approaches result in *yCD*

expression restricted to the period after viral DNA replication. Viral burst size was reduced by less than approximately 10-fold by 5-fluorocytosine (5-FC), showing that expression of yCD as a late gene is compatible with virus replication. Cytopathic effect assays in colon cancer cell lines showed that both yCD viruses have approximately 10-fold increased toxicity in the presence of the prodrug 5-FC, which is converted to 5-fluorouracil (5-FU) by yCD. Toxicity was higher following addition of 5-FC immediately after infection. The largest gain in toxicity was seen in HT29 colon cancer cells, which are the least permissive colon cancer cells for the parental virus, indicating that the new 5-FC/yCD viruses may have broader applications for colon cancer therapy than their predecessors.

- Kaesler, M. D. and R. D. Iggo (2004). "Promoter-specific p53-dependent histone acetylation following DNA damage." **Oncogene** 23: 4007-13 We have used chromatin immunoprecipitation (ChIP) to measure p53-dependent histone acetylation at the p21, MDM2, and PUMA promoters. The pattern of histone acetylation was different at each promoter. H3 and H4 acetylation increased at both the p21 and PUMA promoters in response to p53 activation, whereas there was only a minimal increase in H4 acetylation and no increase in H3 acetylation at the MDM2 promoter. The high p53 occupancy of the p21, MDM2 and PUMA promoters has been attributed to the presence of two p53 binding sites in these promoters, but mutation of the p53 binding sites in integrated p21 promoter constructs showed that the two sites in the p21 promoter do not cooperate to stabilize p53 binding. Despite 10-fold higher p53 binding to the proximal than the distal site in the p21 promoter, both sites showed similar patterns of H3 and H4 acetylation. Mutation of the binding sites showed that acetylation of the proximal, low-affinity site requires p53 binding to that site but not to the distal, high-affinity site. Since low-affinity p53 binding sites can confer strong acetylation, the DNA binding affinity in vitro is an unreliable guide to the likely importance of p53 in regulating candidate target genes in vivo.
- Kaesler, M. D., S. Pebernard and R. D. Iggo (2004). "Regulation of p53 stability and function in HCT116 colon cancer cells." **J Biol Chem** 279: 7598-605 We have used a lentiviral vector to stably express p53 at a physiological level in p53 knockout HCT116 cells. Cells transduced with wild type p53 responded to genotoxic stress by stabilizing p53 and expressing p53 target genes. The reconstituted cells underwent G(1) arrest or apoptosis appropriately depending on the type of stress, albeit less efficiently than parental wild type cells. Compared with cells expressing exogenous wild type p53, the apoptotic response to 5-fluorouracil (5FU) was >50% reduced in cells expressing S15A or S20A mutant p53, and even more reduced by combined mutation of serines 6, 9, 15, 20, 33, and 37 (N6A). Among a panel of p53 target genes tested by quantitative PCR, the gene showing the largest defect in induction by 5FU was BBC3 (PUMA), which was induced 4-fold by wild type p53 and 2-fold by the N6A mutant. Mutation of N-terminal phosphorylation sites did not prevent p53 stabilization by doxorubicin or 5FU. MDM2 silencing by RNA interference activated p53 target gene expression in normal fibroblasts but not in HCT116 cells, and exogenous p53 could be stabilized in HCT116 knockout cells despite combined mutation of p53 phosphorylation sites and silencing of MDM2 expression. The MDM2 feedback loop is thus defective, and other mechanisms must exist to regulate p53 stability and function in this widely used tumor cell line.
- Pebernard, S. and R. D. Iggo (2004). "Determinants of interferon-stimulated gene induction by RNAi vectors." **Differentiation** 72: 103-11 RNA interference is widely used to silence gene expression in mammalian cells. We recently reported that an shRNA expressed from the H1 promoter in a lentiviral vector could induce the expression of a large group of interferon-stimulated genes (ISGs). This response was unrelated to silencing of the gene targeted by the shRNA MORF4L1. In parallel, we constructed lentiviral vectors expressing shRNA from the U6 promoter and found that these too could induce expression of OAS1, a classic interferon target gene. The U6 vectors give a higher frequency of ISG induction than comparable lentiviral H1 vectors, suggesting that there might be a fundamental flaw in the vector design. We have characterized the U6 vectors in detail and report here that ISG induction is a consequence of the presence of an AA di-nucleotide near the transcription start site. A single nucleotide deletion in the siRNA sequence abolished OAS1 induction, suggesting that the mechanism underlying the response uses a sensor that can

detect 19 bp RNA duplexes but not 14 bp duplexes. Adenoviral VA RNA I, which inhibits dsRNA-dependent protein kinase (PKR), was tested as a fusion partner to express shRNA on the grounds that it might prevent nonspecific off-target effects. Fusion of VA RNA I to a lamin shRNA was moderately effective in silencing lamin expression, but gave strong OAS1 induction by an shRNA that does not induce OAS1 when expressed from the U6 or H1 promoters. To avoid interferon induction by U6 vectors, we recommend preserving the wild-type sequence around the transcription start site, in particular a C/G sequence at positions -1/+1, and we describe a simple cloning strategy using the Gateway recombination system that facilitates this task.

Bridge, A. J., S. Pebernard, A. Ducraux, A. L. Nicoulaz and R. Iggo (2003). "Induction of an interferon response by RNAi vectors in mammalian cells." **Nat Genet** 34: 263-4

DNA vectors that express short hairpin RNAs (shRNAs) from RNA polymerase III (Pol III) promoters are a promising new tool to reduce gene expression in mammalian cells. shRNAs are processed to small interfering RNAs (siRNAs) of 21 nucleotides (nt) that guide the cleavage of the cognate mRNA by the RNA-induced silencing complex. Although siRNAs are thought to be too short to induce interferon expression, we report here that a substantial number of shRNA vectors can trigger an interferon response.

Butticaz, C., A. Ciuffi, M. Munoz, J. Thomas, A. Bridge, S. Pebernard, R. Iggo, P. Meylan and A. Telenti (2003). "Protection from HIV-1 infection of primary CD4 T cells by CCR5 silencing is effective for the full spectrum of CCR5 expression." **Antivir Ther** 8: 373-7

Stable gene silencing by RNA interference (RNAi) can be achieved by expression of small hairpin RNAs (shRNAs) from RNA polymerase III promoters. We have tested lentiviral vectors expressing shRNAs targetting CCR5 in primary CD4 T cells from donors representing various CCR5 and CCR2 genetic backgrounds covering the full spectrum of CCR5 expression levels and permissiveness for HIV-1 infection. A linear decrease in CCR5 expression resulted in a logarithmic decrease in cellular infection, giving up to three logs protection from HIV-1 infection in vitro. Protection was maintained at very high multiplicity of infection. This and other recent reports on RNAi should open a debate about the use of RNAi gene therapy for HIV infection.

Chao, C., M. Hergenbahn, M. D. Kaeser, Z. Wu, S. Saito, R. Iggo, M. Hollstein, E. Appella and Y. Xu (2003). "Cell type- and promoter-specific roles of Ser18 phosphorylation in regulating p53 responses." **J Biol Chem** 278: 41028-33 Phosphorylation of mouse p53 at Ser18 occurs after DNA damage. To determine the physiological roles of this phosphorylation event in p53-dependent DNA damage responses, a Ser18 to Ala missense mutation was introduced into the germline of mice. Thymocytes and fibroblasts from the knock-in mice show reduced transactivation of many p53 target genes following DNA damage. p53 protein stabilization and DNA binding are similar in knock-in and wild type mice, but C-terminal acetylation was defective, consistent with a role for Ser18 in the recruitment of transcriptional co-activators. The apoptotic response of knock-in thymocytes to ionizing radiation is intermediate between that of wild type and p53 null thymocytes. Despite impaired transcriptional and apoptotic responses, the knock-in mice are not prone to spontaneous tumorigenesis. This indicates that neither phosphorylation of p53 on Ser18 by ATM nor a full transcriptional response is essential to prevent spontaneous tumor formation in mice.

Malerba, M., L. Daeffler, J. Rommelaere and R. D. Iggo (2003). "Replicating parvoviruses that target colon cancer cells." **J Virol** 77: 6683-91

The wnt signaling pathway is constitutively activated in colon tumors by mutations in the adenomatous polyposis coli and beta-catenin genes. We have modified the minute virus of mice (MVM) P4 promoter to make it responsive to wnt signaling by inserting binding sites for the heterodimeric beta-catenin/Tcf transcription factor. In luciferase assays we can see up to 20-fold selectivity of Tcf mutant P4 promoters for cells with activated wnt signaling. Hybrid MVM/H-1 viruses containing Tcf mutant promoters were tested for NS1 expression, viral DNA replication, virus replication, and cytopathic effect on colon, lung, kidney, and cervical cancer cell lines. Activation of the wnt pathway by expression of Delta N-beta-catenin increased NS1 expression and viral burst size in 293T and H1299 lung cancer cells, showing that the Tcf mutant P4 promoter can respond to wnt signals in the context of the virus. Compared to the parental virus, the

burst size of the Tcf mutant viruses was reduced at least 1,000-fold in H1299, 293T, NB324K, and HeLa cells, which have inactive wnt signaling pathways. The burst size and cytopathic effect of the Tcf viruses was near wild-type levels in SW480 and Isreco1 colon cancer cell lines, which have high Tcf activity. The high specificity of these viruses should permit the development of H-1 virus-based vectors which combine high safety and greater efficacy in cancer therapy.

- Bonnefoi, H., A. Ducraux, S. Movarekhi, M. F. Pelte, S. Bongard, E. Lurati and R. Iggo (2002). "p53 as a potential predictive factor of response to chemotherapy: feasibility of p53 assessment using a functional test in yeast from trucut biopsies in breast cancer patients." **Br J Cancer** 86: 750-5 Assessment of the predictive value of p53 requires the testing of large numbers of samples from patients enrolled in prospective phase III clinical trials. The goal of this study was to determine whether p53 status can be determined by p53 yeast functional assay using the limiting amounts of material that can typically be obtained in prospective phase III trials (particularly when chemotherapy is given before surgery). All patients presenting with a clinically palpable tumour which could be considered large enough to perform a trucut biopsy (> or =2 cm breast tumour) were eligible for this study. Two trucut biopsies and one incisional biopsy were performed on the surgical specimens (mastectomy or tumourectomy). Samples were snap frozen and cryostat sections were taken for histology and p53 testing. Thirty patients were included. Three samples out of 90 failed to give any p53 PCR products, probably because these samples contained almost entirely fibrous tissue. Of the 87 samples that could be tested, the incisional and trucut biopsies results were fully concordant in every case. p53 could be defined in 97% of patients by double trucut biopsy. Eight out of 30 tumours tested were mutant for p53 (27%). p53 status can be reliably determined by yeast assay from single frozen sections of trucut biopsies. Histological examination before p53 testing is essential to exclude cases where the p53 result may reflect only the status of the normal cells in the biopsy.
- Fuerer, C. and R. Iggo (2002). "Adenoviruses with Tcf binding sites in multiple early promoters show enhanced selectivity for tumour cells with constitutive activation of the wnt signalling pathway." **Gene Ther** 9: 270-81 Mutation of the adenomatous polyposis coli and beta-catenin genes in colon cancer leads to constitutive activation of transcription from promoters containing binding sites for Tcf/LEF transcription factors. We have constructed adenoviruses with Tcf binding sites in the early promoters, in order to target viral replication to colon tumours. Tcf regulation of the E1A promoter confers a 100-fold selectivity for cells with activated wnt signalling in viral burst and cytopathic effect assays. p300 is a coactivator for beta-catenin, and E1A inhibits Tcf-dependent transcription through sequestration of p300, but mutation of the p300 binding site in E1A leads to a 10-fold reduction in cytopathic effect of all of the Tcf-regulated viruses. When Tcf sites are inserted in the E1A, E1B, E2 and E4 promoters the viruses show up to 100 000-fold selectivity for cells with activated wnt signalling.
- Kaesler, M. D. and R. D. Iggo (2002). "Chromatin immunoprecipitation analysis fails to support the latency model for regulation of p53 DNA binding activity in vivo." **Proc Natl Acad Sci U S A** 99: 95-100 p53 can adopt two forms in vitro, a latent form that binds naked DNA poorly and an active form that binds DNA well. Conversion of the latent form to the active form is thought to occur by an allosteric mechanism induced by phosphorylation and acetylation. Despite the large differences in affinity produced by regulatory modifications in vitro, mutation of putative regulatory sites has not produced correspondingly large effects on transcription of p53 target genes in vivo. To determine whether genotoxic stress regulates DNA binding by p53 in vivo, we have performed quantitative chromatin immunoprecipitation (ChIP) assays on tumor and normal cell lines containing wild-type p53. ChIP recovers several hundredfold more p21 and MDM2 promoter DNA from p53 wild-type than p53-null cells, indicating that the assay is specific for p53. Genotoxic stress induces much smaller increases in chromatin precipitation, which are matched by changes in the p53 protein level. Thus, in the experimental systems tested, allosteric regulation of DNA binding is not a major level of regulation of p53 activity. The p53 target genes tested can be divided into a group showing high promoter occupancy in vivo (p21, MDM2, and PUMA) and a group giving substantially weaker or background p53 binding (bax, AIP1, and PIG3).

Neither group shows selective recruitment of p53 to the promoter in cells undergoing apoptosis, indicating that the decision to undergo apoptosis or cell cycle arrest depends on other changes in the cell.

- Brunori, M., M. Malerba, H. Kashiwazaki and R. Iggo (2001). "Replicating adenoviruses that target tumors with constitutive activation of the wnt signaling pathway." **J Virol** 75: 2857-65 Despite important advances in understanding the molecular basis of cancer, few treatments have been devised which rationally target known causal oncogenic defects. Selectively replicating viruses have a major advantage over nonreplicating viruses to target these defects because the therapeutic effect of the injected virus is augmented by virus produced within the tumor. To permit rational targeting of colon tumors, we have developed replicating adenoviruses that express the viral E1B and E2 genes from promoters controlled by the Tcf4 transcription factor. Tcf4 is constitutively activated by mutations in the adenomatous polyposis coli and beta-catenin genes in virtually all colon tumors and is constitutively repressed by Groucho and CtBP in normal tissue. The Tcf-E2 and Tcf-E1B promoters are active in many, but not all, cell lines with activation of the wnt pathway. Viruses with Tcf regulation of E2 expression replicate normally in SW480 colon cancer cells but show a 50- to 100-fold decrease in replication in H1299 lung cancer cells and WI38 normal fibroblasts. Activation of wnt signaling by transduction of a stable beta-catenin mutant into normal fibroblasts renders the cells permissive for virus replication. Insertion of Tcf4 sites in the E1B promoter has only small effects on replication in vitro but significantly reduces the inflammatory response in a rodent lung model in vivo. Replicating adenoviruses with Tcf regulation of both E1B and E2 transcription are potentially useful for the treatment of liver metastases from colorectal tumors, but additional changes will be required to produce a virus that can be used to treat all colon tumors.
- Campomenosi, P., P. Monti, A. Aprile, A. Abbondandolo, T. Frebourg, B. Gold, T. Crook, A. Inga, M. A. Resnick, R. Iggo and G. Fronza (2001). "p53 mutants can often transactivate promoters containing a p21 but not Bax or PIG3 responsive elements." **Oncogene** 20: 3573-9 The human p53 protein acts mainly as a stress inducible transcription factor transactivating several genes involved in cell cycle arrest (e.g. p21) or apoptosis (e.g. Bax, PIG3). Roughly half of all human tumours contains p53 missense mutations. Virtually all tumour-derived p53 mutants are unable to activate Bax transcription but some retain the ability to activate p21 transcription. Identification of these mutants may have valuable clinical implications. We have determined the transactivation ability of 77 p53 mutants using reporter yeast strains containing a p53-regulated ADE2 gene whose promoter is regulated by p53 responsive elements derived from the regulatory region of the p21, Bax and PIG3 genes. We also assessed the influence of temperature on transactivation. Our results indicate that a significant proportion of mutants [16/77 (21%); 10/64 (16%) considering only tumour-derived mutants] are transcriptionally active, especially with the p21 promoter. Discriminant mutants preferentially affect less conserved ($P < 0.04$, Fisher's exact test), more rarely mutated ($P < 0.006$, Fisher's exact test) amino acids. Temperature sensitivity is frequently observed, but is more common among discriminant than non-discriminant mutants ($P < 0.003$, Fisher's exact test). Finally, we extended the analysis to a group of mutants isolated in BRCA-associated tumours that surprisingly were indistinguishable from wild type in standard transcription, growth suppression and apoptosis assays in human cells, but showed gain of function in transformation assays. The incidence of transcriptionally active mutations among this group was significantly higher than in the panel of mutants studied previously ($P < 0.001$, Fisher's exact test). Since it is not possible to predict the behaviour of a mutant from first principles, we propose that the yeast assay be used to compile a functional p53 database and fill the gap between the biophysical, pharmacological and clinical fields.
- Tada, M., K. Furuuchi, M. Kaneda, J. Matsumoto, M. Takahashi, A. Hirai, Y. Mitsumoto, R. D. Iggo and T. Moriuchi (2001). "Inactivate the remaining p53 allele or the alternate p73? Preferential selection of the Arg72 polymorphism in cancers with recessive p53 mutants but not transdominant mutants." **Carcinogenesis** 22: 515-7 Several reports have noted epidemiological differences in the prevalence or prognostic significance of p53 mutants with arginine (R) or proline (P) at the codon 72 polymorphism (R72/P72) in certain cancer types, but the biological significance of

these variants is unclear. The ability of p53 mutants to interact with and inactivate the p53 homolog p73 was recently reported to depend on the conformational state of the p53 protein and the residue at codon 72. Since the conformation of p53 mutants may influence their ability to transdominantly inhibit wild-type p53, we tested whether there was a correlation between the amino acid at codon 72 and the transdominance of p53 alleles found in tumors. The transdominance test was performed using a simple yeast transcription assay, and the amino acid at codon 72 was determined by sequencing. A total of 100 p53 mutants were tested. Compared with the germline frequency (R:P = 427:297), an extreme bias in favor of the R72 allele was observed with recessive mutants (R:P = 50:7, $P < 0.0002$), whereas no selection for the R72 allele was seen with transdominant mutants (R:P = 23:20). p53 and p73 are known to transactivate overlapping sets of target genes. We interpret the R72 bias with recessive mutants as evidence that decreased activation of p53 target genes provides a selective growth advantage to tumor cells during the stage of tumorigenesis in which a wild-type and mutant p53 allele coexist. We suggest that transdominant p53 mutants achieve this by inactivation of the remaining wild-type p53 allele, whereas recessive p53 mutants achieve it through inactivation of p73.

Chappuis, P. O., A. Estreicher, B. Dieterich, H. Bonnefoi, M. Otter, A. P. Sappino and R. Iggo (1999). "Prognostic significance of p53 mutation in breast cancer: frequent detection of non-missense mutations by yeast functional assay." **Int J Cancer** 84: 587-93 p53 status was tested in 180 patients with primary breast cancer using a yeast functional assay. Mutations were identified in 32% of cases. Only half were point missense mutations; the remainder were nonsense, insertion, deletion and splice site mutations. Twenty-two percent of mutations were located outside exons 5-8. For a median follow-up of 88 months, survival analysis showed that p53 mutation conferred a worse prognosis in the whole population and the node-positive subgroup but not in node-negative patients. p53 status, tumour size >2 cm, axillary lymph node metastasis and high histological grade were major adverse risk factors in univariate analysis. Multivariate analysis of 153 patients for whom full data were available showed that p53 status contributed prognostic information when tumour size and lymph node status were taken into account but not when histological grade was included. p53 status thus contributes only limited new prognostic information in breast cancer when established prognostic factors are taken into account. *Int. J. Cancer (Pred. Oncol.)* 84:587-593, 1999.

Gagnebin, J., M. Brunori, M. Otter, L. Juillerat-Jeanneret, P. Monnier and R. Iggo (1999). "A photosensitising adenovirus for photodynamic therapy." **Gene Ther** 6: 1742-50 We have developed a new approach to photodynamic therapy based on adenoviral transduction of the rate-limiting enzyme in heme synthesis. Conventional phototherapy uses porphyrin-based chemical photosensitisers, including delta-aminolaevulinic acid (ALA) which is converted to protoporphyrin IX (PpIX) by the enzymes of the heme biosynthetic pathway. The lack of a specific mechanism for targeting chemical photosensitisers and PpIX to tumour cells means that therapeutic irradiation can damage normal tissue and exposure to sunlight following treatment can cause severe burns. The rate limiting enzyme in PpIX synthesis is ALA-synthase (ALA-S). We have developed a new yeast vector system for manipulation of the adeno- virus genome and used it to construct a virus expressing a mutant form of ALA-S lacking the iron response elements which regulate ALA-S translation and the heme regulatory motifs which regulate import of ALA-S into mitochondria. The virus induces a large increase in PpIX expression and confers photosensitivity on cultured cells. Unlike conventional photodynamic therapy, a viral approach makes it possible to restrict photosensitivity by biological rather than purely physical or chemical means. As with HSV thymidine kinase, ALA-S expression is a general mechanism for sensitisation to a therapeutic agent which can easily be adapted to whatever means of gene delivery is most effective.

Saller, E., E. Tom, M. Brunori, M. Otter, A. Estreicher, D. H. Mack and R. Iggo (1999). "Increased apoptosis induction by 121F mutant p53." **Embo J** 18: 4424-37 p53 mutants in tumours have a reduced affinity for DNA and a reduced ability to induce apoptosis. We describe a mutant with the opposite phenotype, an increased affinity for some p53-binding sites and an increased ability to induce apoptosis. The apoptotic function requires transcription activation by p53. The mutant has an altered

sequence specificity and selectively fails to activate MDM2 transcription. Loss of MDM2 feedback results in overexpression of the mutant, but the mutant kills better than wild-type p53 even in MDM2-null cells. Thus the apoptotic phenotype is due to a combination of decreased MDM2 feedback control and increased or unbalanced expression of other apoptosis-inducing p53 target genes. To identify these genes, DNA chips were screened using RNA from cells expressing the apoptosis-inducing mutant, 121F, and a sequence-specificity mutant with the reciprocal phenotype, 277R. Two potential new mediators of p53-dependent apoptosis were identified, Rad and PIR121, which are induced better by 121F than wild-type p53 and not induced by 277R. The 121F mutant kills untransformed MDM2-null but not wild-type mouse embryo fibroblasts and kills tumour cells irrespective of p53 status. It may thus expand the range of tumours which can be treated by p53 gene therapy.

Yuan, Z. Q., N. Wong, W. D. Foulkes, L. Alpert, F. Manganaro, C. Andreutti-Zaugg, R. Iggo, K. Anthony, E. Hsieh, M. Redston, L. Pinsky, M. Trifiro, P. H. Gordon and D. Lasko (1999). "A missense mutation in both hMSH2 and APC in an Ashkenazi Jewish HNPCC kindred: implications for clinical screening." **J Med Genet** 36: 790-3

Flaman, J. M., V. Robert, S. Lenglet, V. Moreau, R. Iggo and T. Frebourg (1998).

"Identification of human p53 mutations with differential effects on the bax and p21 promoters using functional assays in yeast." **Oncogene** 16: 1369-72 Recent studies have suggested that a rare class of p53 mutants found in tumours has a subtle transcriptional defect affecting bax induction but not p21 induction. We have therefore developed simple functional assays in yeast which can be used to identify these mutants. Analysis of 51 different mutations observed in human tumours showed that all mutants tested scored as mutant with the bax reporter strain but nine scored as wild-type with the p21 reporter strain. These results, which can be explained by the lower affinity of the p53 protein for the bax site, may suggest that p21 is not the key target of p53 mutations in tumours. Since p21 status has recently been shown to modulate the chemotherapeutic and radiotherapeutic sensitivities of cancerous cells, the functional assays described here may have important clinical implications.

Gagnebin, J., H. Kovar, A. V. Kajava, A. Estreicher, G. Jug, P. Monnier and R. Iggo (1998).

"Use of transcription reporters with novel p53 binding sites to target tumour cells expressing endogenous or virally transduced p53 mutants with altered sequence-specificity." **Oncogene** 16: 685-90 p53 triple mutants (120N/121G/277H, 120H/121G/277H, 120S/121G/277H and 120H/121G/277Y) have altered sequence specificity in bandshift assays in vitro and transcription assays in vivo. These mutants activate transcription from the site TTT CATG AAA but not from wild type sites. The triple mutants activate more strongly than p53 with a single 277Y mutation. The TTT site matches the wild type p53 consensus at only 4/10 positions and is not recognised by wild type p53. 277Y mutations have been described in human tumours, and Ewing tumour cells expressing this mutant from the endogenous p53 locus selectively activate transcription from transfected luciferase reporters regulated by TTT-mutant p53 binding sites. p53 mutants with altered sequence specificity have potential advantages for cancer gene therapy: if used to activate transcription of conditionally toxic genes they would allow tumour-targeting by p53, which acts as a sensor for the malignant state, but place control over cell killing in the hands of the clinician. Rare tumours expressing such mutants from the endogenous p53 locus could be targeted directly with p53-regulated suicide vectors, but for most tumours both the p53 mutant and the reporter would need to be encoded by the virus.

Tada, M., R. Matsumoto, R. D. Iggo, R. Onimaru, H. Shirato, Y. Sawamura and Y. Shinohe (1998). "Selective sensitivity to radiation of cerebral glioblastomas harboring p53 mutations." **Cancer Res** 58: 1793-7 Recent studies suggest that a balance may exist between the cell cycle arrest and apoptosis-inducing functions of the p53 tumor suppressor gene. Adenoviral p21 transduction attenuates apoptosis, whereas deletion of the p21 gene promotes it, and p21-null xenografts respond better than isogenic p21-wild type tumors to irradiation. Hence, the role of p53 in dictating the clinical response to radiotherapy and chemotherapy may be more complex than previously thought. We have analyzed survival and radiation response (regrowth-free period) of 42 patients with glioblastomas whose p53 status was determined by a sensitive yeast functional assay. Multivariate analysis revealed that p53 mutation is

associated with longer survival ($P < 0.02$). Among 36 radiation-treated patients, the regrowth-free period after treatment was significantly longer for tumors with p53 mutations ($P < 0.0001$), and p53 mutation was the sole independent factor predictive of radiotherapeutic response ($P < 0.01$). Survival time after regrowth was independent of p53 status, suggesting that the difference in survival was related to the treatment rather than to the intrinsic aggressiveness of the tumor. Thus, in this Northern Japanese population, p53 mutation is a marker for better radiation response in glioblastomas, and this results in significantly longer survival.

- Andreutti-Zaugg, C., R. J. Scott and R. Iggo (1997). "Inhibition of nonsense-mediated messenger RNA decay in clinical samples facilitates detection of human MSH2 mutations with an in vivo fusion protein assay and conventional techniques." **Cancer Res** 57: 3288-93
- Germ-line mutations in the human MSH2 (hMSH2) gene account for about 40% of known defects in kindreds with hereditary nonpolyposis colon cancer. We describe a simple fusion protein assay for detection of hMSH2 nonsense mutations in yeast. Detection of nonsense mutations with this assay is severely compromised in many cases by nonsense-mediated mRNA decay, a physiological process that destabilizes the mutant RNA. Triggering of nonsense-mediated decay requires mRNA scanning by the ribosome to detect the stop codon. We show that treatment of cells with the translation inhibitor puromycin suppresses nonsense-mediated decay and facilitates the detection of nonsense mutations in clinical samples by cDNA sequencing, in vitro protein truncation tests, and the yeast fusion protein assay. Given the prevalence of chain-terminating mutations in human disease genes, puromycin treatment of blood samples should improve the signal-to-noise ratio and hence the sensitivity of many RNA-based diagnostic tests. Paradoxically, the yeast hMSH2::ADE2 fusion protein assay also detects some in-frame mutations, presumably through an effect on the folding of the fusion protein.
- Inga, A., S. Cresta, P. Monti, A. Aprile, G. Scott, A. Abbondandolo, R. Iggo and G. Fronza (1997). "Simple identification of dominant p53 mutants by a yeast functional assay." **Carcinogenesis** 18: 2019-21
- Analysis of families with germline p53 mutations shows that the mutant p53 allele behaves as a dominant oncogene at the genetic level, although it behaves as a recessive oncogene at the cellular level, since tumours invariably show mutation or loss of both wild-type alleles. At the biochemical level it is possible that some clinically important mutant p53 proteins may be carcinogenic through a dominant mechanism. We show that p53 mutants can be readily classified according to their dominant potential using a simple yeast functional assay. Wild-type p53 is constitutively expressed from a TRP1 vector, p53 mutants are expressed from an otherwise identical LEU2 vector and net transcriptional activity is scored using an ADE2-based reporter. Twenty seven p53 mutants were tested: 19 were recessive, i.e. gave white colonies, and eight showed dominant activity, i.e. gave pink/red colonies. This simple assay should facilitate studies on p53 dominance.
- Inga, A., R. Iannone, P. Monti, F. Molina, M. Bolognesi, A. Abbondandolo, R. Iggo and G. Fronza (1997). "Determining mutational fingerprints at the human p53 locus with a yeast functional assay: a new tool for molecular epidemiology." **Oncogene** 14: 1307-13
- In order to isolate experimentally induced p53 mutations, a yeast expression vector harbouring a human wild-type p53 cDNA was treated in vitro with the antineoplastic drug chloroethyl-cyclohexyl-nitroso-urea (CCNU) and transfected into a yeast strain containing the ADE2 gene regulated by a p53-responsive promoter. p53 mutations were identified in 32 out of 39 plasmids rescued from independent ade-transformants. Ninety-two percent of CCNU induced mutations were GC-targeted single base pair substitutions, and GC > AT transitions represented 73% of all single base pair substitutions. In 70% of the cases the mutated G was preceded 5' by a purine. The distribution of the mutations along the p53 cDNA was not random: positions 734 and 785 appeared as CCNU mutational hotspots ($n=3$, $P<0.0003$) and CCNU induced only GC > AT transitions at those positions. The features of these CCNU-induced mutations are consistent with the hypothesis that O6-alkylguanine is the major causative lesion. One third of the CCNU-induced mutants were absent from a huge collection of 4496 p53 mutations in human tumours and cell lines, thus demonstrating that CCNU has a mutational spectrum which is uniquely different from that of naturally selected mutations. This strategy allows direct comparison of

- observed natural mutation spectra with experimentally induced mutation spectra and opens the way to a more rigorous approach in the field of molecular epidemiology.
- Waridel, F., A. Estreicher, L. Bron, J. M. Flaman, C. Fontolliet, P. Monnier, T. Frebourg and R. Iggo (1997). "Field cancerisation and polyclonal p53 mutation in the upper aero-digestive tract." **Oncogene** 14: 163-9 Field cancerisation of the aerodigestive tract is caused by chronic exposure to alcohol and tobacco, but the nature of the genetic alterations preceding overt malignancy is unknown. To identify potential field changes we have used a functional assay which tests the transcriptional competence of human p53 expressed in yeast. To increase the sensitivity and reliability of the technique for samples containing under 20% mutant p53, the 5' and 3'-ends of the p53 cDNA were examined separately. With this split form of the assay the tissue p53 mRNA acts as its own control for RNA quality. Mutations were detected in 87% (46/53) of tumours, reflecting the high sensitivity of the technique. Multiple biopsies of histologically normal tissue from the upper aero-digestive tract were tested and clonal p53 mutations were identified in 76% (38/50) of biopsies from patients presenting with multiple tumours compared with 32% (38/117) of biopsies from patients presenting with single tumours ($P < 0.000001$). All patients (16/16) presenting with multiple tumours had at least one positive biopsy, compared with only 53% (19/36) of patients presenting with single tumours ($P < 0.001$). This defines expansion of multiple clones of mutant p53-containing cells as an important biological mechanism of field cancerisation, and provides a means to identify patients likely to benefit from intensive screening for the development of new head and neck tumours.
- Cottu, P. H., F. Muzeau, A. Estreicher, J. F. Flejou, R. Iggo, G. Thomas and R. Hamelin (1996). "Inverse correlation between RER+ status and p53 mutation in colorectal cancer cell lines." **Oncogene** 13: 2727-30 The high point mutation rate of replication error-prone (RER+) cells could theoretically lead to inactivation of the p53 gene by polyclonal mutations, which might explain the conflicting results that have been published on the p53 status of RER+ colon cancers. To address this issue, we tested the p53 status of 21 human colorectal cancer cell lines, including four showing microsatellite instability (RER+ phenotype). Denaturing gradient gel electrophoresis (DGGE) followed by sequencing showed that all four RER+ cell lines were wild type for p53 while 15 of the 17 RER- cell lines contained p53 mutations ($P = 0.001$). Eight cell lines (four RER+ and four RER-) were analysed using three complementary methods to test more rigorously the polyclonal mutation hypothesis. (i) Of 87 single-cell clones (seven to 14 per cell line) examined by DGGE, only those derived from known p53 mutant cell lines showed altered profiles. (ii) Antibody DO-7 stained more than 80% of nuclei from the p53 mutant cell lines, but only 15% of nuclei from the RER+ cell lines. (iii) A yeast functional assay which can simultaneously detect polyclonal mutations at over 500 different sites in the p53 cDNA scored all four RER+ cell lines as containing only transcriptionally active p53. These data thus do not support the polyclonal mutation hypothesis and instead suggest that mismatch repair deficiency provides a p53-independent pathway for development of colorectal cancers.
- Flaman, J. M., F. Waridel, A. Estreicher, A. Vannier, J. M. Limacher, D. Gilbert, R. Iggo and T. Frebourg (1996). "The human tumour suppressor gene p53 is alternatively spliced in normal cells." **Oncogene** 12: 813-8 Alternative splicing affecting the p53 carboxy-terminus has previously been described in mouse but not in normal human cells. We report here the detection in normal human lymphocytes of an alternatively spliced form of human p53 mRNA containing an additional 133 bp exon derived from intron 9. This splice variant encodes a truncated protein of 341 amino-acids including 10 new amino-acids derived from the novel exon. The truncated protein, which lacks part of the p53 tetramerization domain, fails to bind DNA in vitro and has a transcriptional defect in vivo in both yeast and mammalian cells. Quantitative RT-PCR experiments suggest that the alternatively spliced form is only present in significant amounts in quiescent cells. Considering the numerous functions ascribed to the carboxy-terminus of the p53 protein, this splice variant may have important implications for the biological role of p53 in normal cells.
- Guillou, L., A. Estreicher, P. Chaubert, J. Hurlimann, A. M. Kurt, G. Metthez, R. Iggo, A. C. Gray, P. Jichlinski, H. J. Leisinger and J. Benhattar (1996). "Germ cell tumors of the testis overexpress wild-type p53." **Am J Pathol** 149: 1221-8 Several recent

studies have suggested that testicular germ cell tumors express high levels of wild-type p53 protein. To clarify and confirm this unexpected result, we have investigated seminomatous and nonseminomatous germ cell tumors at the genomic, mRNA, and protein levels. Thirty-five tumors were examined for p53 overexpression using antibodies directed against the p53 (PAb1801, PAb240, and CM1), mdm2 (IF2), and p21Waf1/Cip1 (EA10) proteins. Thirty-two tumors were screened for p53 mutations by single-strand conformation polymorphism analysis. Eighteen tumors were screened with a functional assay that tests the transcriptional competence of human p53 protein expressed in yeast. On frozen sections, 100, 65, 35, 73, and 0% of tumors reacted with the CM1, PAb240, PAb1801, IF2, and EA10 antibodies, respectively. No p53 mutations were detected by single-strand conformation polymorphism or by functional assay. The fact that many tumors overexpress wild-type p53 but not mdm2 rules out mdm2 overexpression as a general explanation for the presence of wild-type p53 in these tumors. The absence of p21 overexpression suggests that p53 may be unable to activate transcription of critical target genes, which may explain why the presence of wild-type p53 is tolerated in this tumor type, although the mechanism for this transcriptional inactivity remains to be established.

Shaw, P., J. Freeman, R. Bovey and R. Iggo (1996). "Regulation of specific DNA binding by p53: evidence for a role for O-glycosylation and charged residues at the carboxy-terminus." **Oncogene** 12: 921-30 The carboxy-terminus of p53 contains a basic region which represses DNA binding, and this repression can be relieved by PAb421, an antibody against the basic region. The EB-1 human cell line contains wild type p53 protein which fails to express the PAb421 epitope and is highly active both in biological assays and in DNA binding assays. We show by wheat germ agglutinin chromatography and galactosyl-transferase labelling that this p53 is O-glycosylated, and that at least one of the sugar residues masks the PAb421 epitope, as demonstrated by recovery of reactivity with PAb421 after digestion of Western blots of EB-1 cell extract with hexosaminidase. A minor population of p53 molecules in EB-1 cells lacks the modification, and there is a correlation between the ability to bind DNA with high affinity and masking of the PAb421 epitope. We also show that strongly positively charged peptides, including short peptides from the basic region of p53, can derepress DNA binding, probably by disruption of an intramolecular interaction involving the basic region. We propose that any intervention which prevents this intramolecular interaction, including addition of bulky residues such as sugar groups, can activate DNA binding by p53.

Barta, I. and R. Iggo (1995). "Autoregulation of expression of the yeast Dbp2p 'DEAD-box' protein is mediated by sequences in the conserved DBP2 intron." **Embo J** 14: 3800-8

The human p68, *Saccharomyces cerevisiae* DBP2 and *Schizosaccharomyces pombe* dbp2 genes are closely related members of the 'DEAD-box' RNA helicase superfamily. All three genes contain an intron at a conserved site in RNA helicase motif V. The *S.cerevisiae* intron is unusual both for its position near the 3'-end of the open reading frame and for its size, 1001 nucleotides. We show here that precise deletion of the intron has no effect on cell viability but leads to an increase in Dbp2p protein expression. Inefficient splicing due to the size of the intron can not account for this difference because the intron is efficiently spliced in Dbp2p-deficient cells. Instead, there is a reciprocal relationship between the amount of Dbp2p in the cell and the efficiency with which DBP2 intron-containing genes are expressed. Inactive Dbp2p mutants are efficiently expressed from DBP2 intron-containing plasmids, and fragments of the DBP2 intron confer Dbp2p-responsiveness on heterologous reporter introns. This suggest that there is an intron-mediated negative feedback loop regulating DBP2 expression, and provides a possible explanation for the retention of such an unusual intron in *S.cerevisiae*.

Flaman, J. M., T. Frebourg, V. Moreau, F. Charbonnier, C. Martin, C. Ishioka, S. H. Friend and R. Iggo (1994). "A rapid PCR fidelity assay." **Nucleic Acids Res** 22: 3259-60

Freeman, J., S. Schmidt, E. Scharer and R. Iggo (1994). "Mutation of conserved domain II alters the sequence specificity of DNA binding by the p53 protein." **Embo J** 13: 5393-400 We have mutagenized human p53 expressed in yeast and selected two mutants, 121F and 123A, which activate transcription from one, rather than the normal two, copies of the consensus p53 DNA binding sequence. Both mutants have

a 6-fold increase in affinity for a single copy of the sequence GGG CATG CCC. The 121F mutant has a decrease, and the 123A mutant an increase, in the affinity for the sequence GAA CATG TTC. This genetic and biochemical evidence supports the crystallographic finding that amino acid 120 contacts guanine in the major groove at the second position in the consensus. The major p53 binding site in the p21WAF1/CIP1 promoter resembles the GAA CATG TTC form of the consensus. Compared with wild type p53, the 121F mutant has a 7-fold lower affinity for the p21WAF1/CIP1 site in vitro, and the 121F mutant is defective in p21 induction in vivo. Mutants with subtly altered sequence specificity may facilitate dissection of downstream pathways activated by p53.

- Ishioka, C., T. Frebourg, Y. X. Yan, M. Vidal, S. H. Friend, S. Schmidt and R. Iggo (1993). "Screening patients for heterozygous p53 mutations using a functional assay in yeast." **Nat Genet** 5: 124-9 Inherited mutations of the p53 gene significantly increase the risk of developing diverse malignancies, and germline p53 mutations can be detected by assaying the transcriptional activity of the p53 protein in mammalian cells. Here we describe a method starting with lymphocytes that allows detection of germline p53 mutations by 'functional' analysis of p53 protein expressed in *Saccharomyces cerevisiae*. The p53 PCR products are directly cloned into yeast expression vectors in vivo and subsequently tested for transcriptional activity in a simple growth assay. This technique, functional analysis of separated alleles in yeast (FASAY), requires only a few steps, can be automated readily and should permit screening for germline or somatic heterozygous mutations in any gene whose function can be monitored in yeast.
- Scharer, E. and R. Iggo (1992). "Mammalian p53 can function as a transcription factor in yeast." **Nucleic Acids Res** 20: 1539-45 p53 has previously been shown to contain a transactivation domain using GAL4 fusion proteins and to bind specifically to a 33 base pair DNA sequence in immunoprecipitation assays. We show here that mammalian p53 expressed in *S. cerevisiae* is able to activate transcription of a reporter gene placed under the control of a CYC1 hybrid promoter containing the 33 base pair p53-binding sequence. The activation is dependent on the orientation and number of copies of the binding site. Three p53 mutants commonly found in human tumours, 175H, 248W and 273H, are unable to activate transcription. A fourth human p53 mutant, 285K, is temperature-sensitive for transcriptional activation. Murine p53 activates transcription from the same sequence. The murine 135V mutant, which is temperature-sensitive for mammalian cell transformation, is also temperature-sensitive for transcriptional activation. There is a much better correlation between mutation and transcriptional competence than between mutation and the structure of p53 determined with conformation-sensitive antibodies. We have therefore developed a simple transcription assay for p53 mutation in which yeast are transfected with p53 PCR products and mutation is scored on X-gal plates.
- Iggo, R. D., D. J. Jamieson, S. A. MacNeill, J. Southgate, J. McPheat and D. P. Lane (1991). "p68 RNA helicase: identification of a nucleolar form and cloning of related genes containing a conserved intron in yeasts." **Mol Cell Biol** 11: 1326-33 The human p68 protein is an RNA-dependent ATPase and RNA helicase which was first identified because of its immunological cross-reaction with a viral RNA helicase, simian virus 40 large T antigen. It belongs to a recently discovered family of proteins (DEAD box proteins) that share extensive regions of amino acid sequence homology, are ubiquitous in living organisms, and are involved in many aspects of RNA metabolism, including splicing, translation, and ribosome assembly. We have shown by immunofluorescent microscopy that mammalian p68, which is excluded from the nucleoli during interphase, translocates to prenucleolar bodies during telophase. We have cloned 55% identical genes from both *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* and shown that they are essential in both yeasts. The human and yeast genes contain a large intron whose position has been precisely conserved. In *S. cerevisiae*, the intron is unusual both because of its size and because of its location near the 3' end of the gene. We discuss possible functional roles for such an unusual intron in an RNA helicase gene.
- Bartek, J., R. Iggo, J. Gannon and D. P. Lane (1990). "Genetic and immunochemical analysis of mutant p53 in human breast cancer cell lines." **Oncogene** 5: 893-9 The expression of the tumour suppressor gene p53 was analysed in 11 human breast

cancer cell lines by immunohistochemistry, immunoprecipitation and cDNA sequencing. We used a panel of anti-p53 monoclonal antibodies for cell staining and found abnormalities in every case. Eight of the cell lines produce a form of p53 which can be immunoprecipitated by the monoclonal antibody PAb240 but not by PAb1620. In the murine system PAb240 only immunoprecipitates mutant p53. We sequenced p53 cDNA directly from four of the PAb240 positive cell lines using asymmetric PCR templates. All four contained missense mutations in p53 RNA, with no detectable expression of the wild type sequence. Different residues were affected in each cell line, but all the mutations changed amino acids conserved from man to Xenopus. These results imply that as in the murine system, the PAb240 antibody reliably detects a wide variety of p53 mutations and that these mutations have a common effect on the structure of p53. Immunohistochemical data suggest that p53 mutation is the commonest genetic alteration so far detected in primary breast cancer.

Gannon, J. V., R. Greaves, R. Iggo and D. P. Lane (1990). "Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form." **Embo J** 9: 1595-602 Point mutations in the p53 gene are the most frequently identified genetic change in human cancer. They convert murine p53 from a tumour suppressor gene into a dominant transforming oncogene able to immortalize primary cells and bring about full transformation in combination with an activated ras gene. In both the human and murine systems the mutations lie in regions of p53 conserved from man to Xenopus. We have developed a monoclonal antibody to p53 designated PAb240 which does not immunoprecipitate wild type p53. A series of different p53 mutants all react more strongly with PAb240 than with PAb246. The PAb240 reactive form of p53 cannot bind to SV40 large T antigen but does bind to HSP70. In contrast, the PAb246 form binds to T antigen but not to HSP70. PAb240 recognizes all forms of p53 when they are denatured. It reacts with all mammalian p53 and chicken p53 in immunoblots. We propose that immunoprecipitation of p53 by PAb240 is diagnostic of mutation in both murine and human systems and suggest that the different point mutations which convert p53 from a recessive to a dominant oncogene exert a common conformational effect on the protein. This conformational change abolishes T antigen binding and promotes self-oligomerization. These results are consistent with a dominant negative model where mutant p53 protein binds to and neutralizes the activity of p53 in the wild type conformation.

Iggo, R., K. Gatter, J. Bartek, D. Lane and A. L. Harris (1990). "Increased expression of mutant forms of p53 oncogene in primary lung cancer." **Lancet** 335: 675-9
Primary lung cancer samples of the major histological types were examined for expression of the tumor suppressor gene p53 by immunohistochemistry. Abnormalities in p53 expression were found in 28 of 40 carcinomas, 14 of 17 squamous tumours showing abnormal p53 expression, whereas no expression of p53 was detectable in 7 carcinoid tumours or in 10 normal lung samples. Direct evidence for homozygous expression of mutant p53 mRNA in representative carcinomas was obtained by means of an asymmetric polymerase chain reaction mRNA sequencing strategy, which allowed sequencing without any cloning step. All the mutations were G to T transversions resulting in mis-sense mutations in aminoacids highly conserved in evolution. Mutation of the p53 gene is the most frequently identified genetic change in human lung cancer; these findings suggest that simple immunohistological methods can provide strong evidence of such mutation.

Iggo, R., S. Picksley, J. Southgate, J. McPheat and D. P. Lane (1990). "Identification of a putative RNA helicase in E.coli." **Nucleic Acids Res** 18: 5413-7 The human p68 protein, an SV40 large T related antigen, is an RNA dependent ATPase and RNA helicase. It belongs to a new large and highly conserved gene family, the DEAD box proteins, whose members are involved in a variety of processes requiring manipulation of RNA secondary structure such as translation and splicing. Multiple DEAD box genes are present in S.cerevisiae, but only one has previously been described in E.coli. Low stringency screening of an E.coli genomic library with a p68 cDNA probe led to the identification of dbpA, a new E.coli DEAD box gene located at 29.6 minutes on the W3110 chromosome. We report here the nucleotide and deduced amino acid sequences of the gene. We have overexpressed dbpA from its

own promoter on a high copy number plasmid and identified the gene product as a approximately 50 kD protein by immunoblotting with an anti-DEAD antibody.

- Iggo, R., A. Gough, W. Xu, D. P. Lane and N. K. Spurr (1989). "Chromosome mapping of the human gene encoding the 68-kDa nuclear antigen (p68) by using the polymerase chain reaction." **Proc Natl Acad Sci U S A** 86: 6211-4 Chromosome mapping by Southern analysis of DNA from somatic cell hybrids is often unsuccessful when only cDNA probes are available. p68 is a putative RNA helicase that is antigenically related to the simian virus 40 large tumor antigen. By using the polymerase chain reaction and oligode-oxynucleotide primers based on the cDNA sequence, we have identified introns in the p68 gene. Comparison of human and mouse DNA fragments amplified with these primers revealed length differences that allowed us to identify the human gene. Application of this technique to DNA from human-mouse somatic cell hybrids and cell lines derived from them by chromosome-mediated gene transfer allowed us to map p68 to the distal part of the long arm of chromosome 17.
- Iggo, R. D. and D. P. Lane (1989). "Nuclear protein p68 is an RNA-dependent ATPase." **Embo J** 8: 1827-31 The human nuclear antigen p68 cross reacts with a monoclonal antibody to SV40 large-T antigen. Its deduced amino acid sequence contains short motifs which place it in a large superfamily of proteins of known or putative helicase activity. Recently, a p68 subfamily (DEAD box proteins) which share more extensive regions of homology has been identified in mouse, *Drosophila*, *Saccharomyces cerevisiae* and *Escherichia coli*. These proteins are involved in translation, ribosome assembly, mitochondrial splicing, spermatogenesis and embryogenesis. We show here that immunopurified human p68 has RNA dependent ATPase activity. In addition, we show that the protein undergoes dramatic changes in cellular location during the cell cycle.
- Mole, S. E., R. D. Iggo and D. P. Lane (1989). "Using the polymerase chain reaction to modify expression plasmids for epitope mapping." **Nucleic Acids Res** 17: 3319