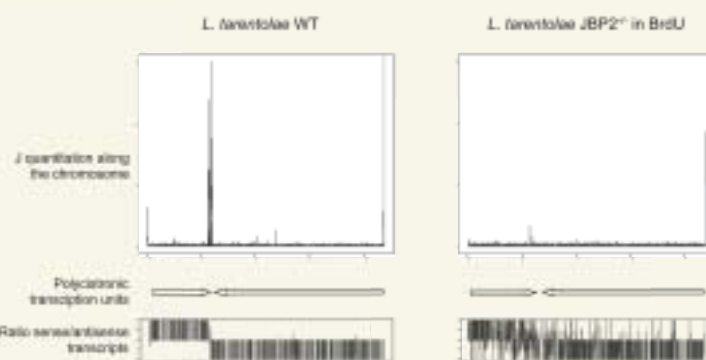


DNA BASE J

-glucosyl-hydroxymethyluracil (base J), which we discovered in African trypanosomes in 1993 (Gommers-Ampt *et al.*, Cell 1993;75:1129-1136), is a base present in kinetoplastid flagellates and in *Euglena*. It replaces 1% of thymine in nuclear DNA and is predominantly located in repetitive sequences, such as telomeric repeats. We have characterized a J-binding protein (JBP1) that binds to J-containing duplex DNA (Cross *et al.* EMBO J 1999;18:6573-6581). We have shown that JBP1 is a thymidine hydroxylase that catalyses the first step of J biosynthesis, the conversion of T in DNA into hydroxymethyluracil. JBP1 belongs to the family of Fe²⁺ and 2-oxoglutarate-requiring dioxygenases, as does a second putative hydroxylase, JBP2. In the kinetoplastid *Leishmania*, a JBP1 KO is lethal. In contrast, JBP2 is dispensable in *Leishmania*, but JBP2 KO strains are hypersensitive to bromodeoxyuridine (BrdU). During growth in BrdU, *Leishmania* loses its J, which is located for > 98% in telomeric repeats in this organism. How J loss leads to cell death is unclear. We do not find alterations in DNA integrity or cell cycle blocks. A recent breakthrough came from the discovery by R. Sabatini (University of Georgia, US) that trypanosomes have J at transcriptional start and stop sites. Using immuno-precipitation of J-DNA and deep sequencing, we have also found the 1% of non-telomeric J in *Leishmania* at specific chromosome-internal positions, partly at transcriptional stops (collaboration with NKI-AVL deep sequencing unit and Peter Myler, Seattle). We have shown that loss of this internal J leads to massive readthrough of RNA Polymerase II transcriptional stops, as shown in figure 2, suggesting that transcriptional termination is a major function of J. We have also found an interesting interaction between J and histone H3/H3V (H3 variant) and the tools are on board to determine the role of the chromatin environment in J function. With Anastassis Perrakis (NKI-AVL) we are trying to determine the structure of JBP1-J-DNA complexes by crystallography. In 2010 the structure of the DNA-binding domain of JBP1 was solved. Interestingly, this domain has a unique structure not seen before in DNA-binding proteins and the specific binding of JBP1 to J-DNA was shown to be dependent on a single aspartate residue interacting with the glucose-moiety of base J.

Figure 2:
Readthrough of
RNA Polymerase
II transcription
termination stops
upon loss of J
in *Leishmania*
tarentolae. With a
ChIP experiment
we determined the
distribution of J
along chromosome

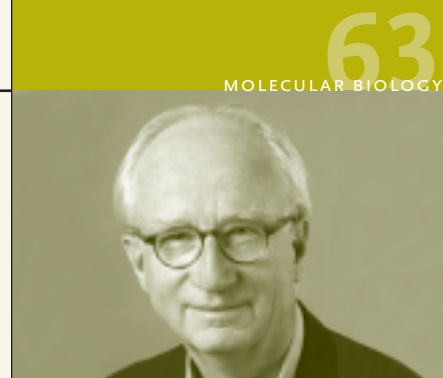


4. Internal J is located at the transcription termination site of the two long convergent polycistronic transcription units. J is lost from this location in the JBP1KO cell line grown in the presence of BrdU. We sequenced and mapped the small RNA degradation products present in WT and mutant cell lines and plotted the ratio of sense/antisense transcript. When J is lost, antisense transcripts spread from the transcription stop region to both sides of the chromosome due to readthrough of the stop site.

DRUG TRANSPORTERS

We are interested in mechanisms of drug resistance in cancer cells and focus on resistance caused by increased ATP-dependent transport of drug out of the cell, mediated by ATP-binding cassette (ABC) transporters. We have isolated genes for these transporters and are characterizing their substrate specificity and sensitivity to inhibitors in transfected cells. To study the physiological function of these transporters, we have inactivated genes for several drug transporters by targeted gene disruption in mice. We are mainly studying the Multidrug Resistance Protein (ABCC) family members MRP2, 3, 4, 5 and 6.

We have initiated a systematic search for compounds conjugated to glucuronide



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Publications

Beedholm-Ebsen R, Van de Wetering K, Hardlei T, Nexø E, Borst P, Moestrup SK. Identification of multidrug resistance protein 1 (MRP1/ABCC1) as a molecular gate for cellular export of cobalamin. *Blood* 2010;115:1632-9

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or sulphate that are transported by MRPs by comparing the derivatives in plasma/urine of WT and KO mice using Mass Spectrometry (MS). We have identified several glucuronidated and sulphated phyto-estrogens, derived from food, as novel MRP2 and MRP3 substrates by this approach. In addition, we have found that conjugated retinoids are MRP2 substrates. We are refining the LC/MS analysis to allow the identification of all compounds altered in plasma/urine of KO mice. This approach should also be helpful in finding substrates of other MRPs and BCRP (ABCG2).

DRUG RESISTANCE IN 'SPONTANEOUS' MOUSE TUMORS

In collaboration with Jos Jonkers (NKI-AVL), we are studying resistance mechanisms in "spontaneous" mammary tumors arising in mice, conditionally defective in *p53* and *Brca1*. When treated with the maximum tolerable dose of doxorubicin, docetaxel or topotecan, the breast tumors initially respond but eventually always develop resistance. Resistance is often associated with upregulation of the *Mdr1a* and *Mdr1b* genes (*Abcb1*), which encode drug-transporting P-glycoproteins (P-gps) and we have shown with specific inhibitors that remarkably low levels of *Abcb1* upregulation (5-fold the levels in sensitive tumors) suffice to make the tumor multidrug resistant. We are also using this mouse model to test new anticancer drugs and drug combinations. Impressive tumor regression has been obtained with a new inhibitor of Poly-ADP-ribose polymerase I (PARPI), olaparib, but resistance to this compound also arises by *Abcb1* upregulation. We have crossed disrupted alleles for the *Abcb1* genes into our mouse model to further test the importance of these transporters in drug resistance and to uncover other forms of resistance, - notably to doxorubicin, docetaxel and olaparib -, not mediated by P-gp.

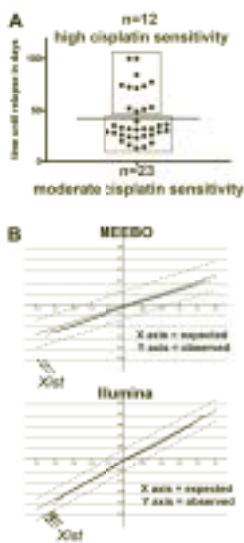
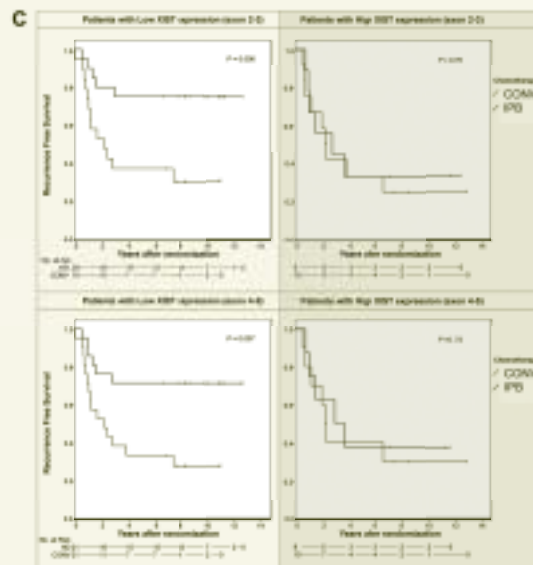


Figure 3: Correlation of gene expression with the response to platinum drugs. (A) 35 mice with *Brca1*^{-/-};*p53*^{-/-} mammary tumors were treated with 6mg of cisplatin per kg i.v. on day 0. The time until relapse is the time required for the tumors to grow back to 100%. (B) Significance analysis of microarrays of highly versus moderately cisplatin-sensitive *Brca1*^{-/-};*p53*^{-/-} mouse mammary tumors using the MEEBO ($D=1.5$; $FDR=0$) or Illumina ($D=0.85$; $FDR=0$) platform. (C) Kaplan Meier survival curves according to *XIST* gene expression levels of patients with HER2-negative, high-risk breast cancer who had been randomized between conventional (CONV, grey) and intensive platinum-based chemotherapy (IPB, black). P values were calculated using the logrank test.



In contrast to the results obtained with MDR drugs, we have been unable to obtain cisplatin resistance in this tumor model. The tumors respond to each new treatment with cisplatin, but are never fully eradicated. Although we have identified a tumor-initiating cell ("stem cell") in this tumor model characterized by high surface expression of CD24 and CD49f, this fraction does not appear to be enriched in the "remnants" from which the tumors regrow after chemotherapy. We are testing the hypothesis that the resistance of "remnants" is not due to specific biochemical defense mechanisms of the putative tumor stem cells, but to the ability of a sub-fraction of the cells to go into "hibernation", i.e., stop cell cycle progression until the drug is gone and the DNA damage has been repaired. We have succeeded in isolating tumor cell lines in low O₂ that resemble the original tumor. These cultured cells have allowed us to study the fraction of cells surviving cisplatin. Remarkably, this fraction does not contain G₂/M cells, but appears to stall in G₁/G₀. We are studying how these cells escape cisplatin-induced death. The initial response to drug treatment of these tumors varies from tumor to tumor. We can distinguish "good" from "poor" responders. As this tumor model is genetically so homogeneous, it is in principle ideal for identifying markers that predict drug response. We have shown in our model that standard gene expression profiling is unable to detect drug resistance mechanisms that cause poor drug response in a minority of the tumors. This explains the failure of this approach in the clinic. We have found, however, low *Xist* expression in most mouse tumors with "good" initial response to cisplatin (figure 3). Low *Xist* expression was subsequently also found in our clinic to predict long recurrence-free survival in a group of breast cancer patients treated with platinum-based chemotherapy.