EZ RT-PCR (PE Applied Biosystems, Foster City, CA) using primers and probes specific for the 5' and the 3' regions of GAPDH. **RESULTS.** A549 and primary breast epithelial cells fixed in RNA*later*™ showed good staining patterns with DNA dyes and CAM 5.2 cytokeratin antibodies. Although fixation was effective after 1 hour in the reagent, cytokeratin fluorescence intensity was further enhanced and DNA content CVs were improved by continued incubation of cells for longer periods of time. Visual observation by fluorescence microscopy showed that epithelial cells were intact after sorting at a rate of 2–3 x 10^3 events/sec. Equivalent amounts of total RNA (3–10 ug/ 10^6 cells) were isolated from fixed sorted and fixed unsorted epithelial cells. The ratios of real-time PCR threshold concentrations (C_T) for the GAPDH probes (3'/5' C_T) were equivalent for control RNA, and for RNA from unsorted and sorted epithelial cells fixed and stained in RNA*later*™. Cells fixed in EtOH/acetic acid had poorer mRNA yields and lower C_T ratios. Furthermore, whereas scrupulous cleaning of the cytometer tubing and use of the RNAse inhibitor DEPC in sheath fluid was required in order to preserve message in EtOH/acetic acid fixed cells; such treatment was not required for cells fixed in RNA*later*™. Agarose gel analysis showed that high C_T values correlated with intact 28S and 18S rRNA bands. DNA extracted simultaneously with RNA from sorted epithelial cells provided a template for whole genome amplification, genotyping and sequencing. **CONCLUSIONS**. The aqueous reagent, RNA*later*™, which fixes and preserves RNA, allows staining and purification of whole epithelial cells by flow sorting. High yields of RNA and DNA suitable for expression and genotype analysis can be obtained from flow cytometrically purified populations of neoplastic cells from tissues *in vivo*.

Becker, Kevin

Identifying biological pathway information from cDNA arrays: BBID, the biological biochemical image database

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Complex biological regulatory pathways of higher eukaryotes are poorly described, difficult to present and difficult to study. One reason for this is a lack of a central repository for regulatory pathway information. The Biological Biochemical Image Database (BBID) is a World-Wide Web searchable database of images from research articles that describe regulatory pathways of higher eukaryotes. These images are not redrawn, were obtained with permission, are referenced and are thus attributable back to the original journal and authors. The elements of each image are loaded into a table that can be searched by keywords including: gene name, cell/tissue type, species etc. In parallel, these image elements are attached to the gene files that underlie large-scale gene expression studies such as cDNA microarrays. Pathway information for complex gene expression studies can then be extracted automatically. In this way, complex regulatory pathways can be tested empirically in an efficient manner in the context of large-scale gene expression systems. The BBID can be accessed at the following address http://www.grc.nia.nih.gov/hd/bbid99.htm.

Bjorbaek, Christian

Identification of novel hypothalamic genes that are regulated by leptin and play a role in development of leptin-resistance and obesity

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Background. Leptin, a critical hormone produced in adipose tissue, provides information on energy stores and energy balance to brain centers that regulate appetite, energy expenditure and neuroendocrine function[1,2]. Leptin acts on leptin receptors expressed in regions of the hypothalamus to activate signal transduction [3,4,5] and to regulate mRNA expression of key hypothalamic neuropeptides, some well known (NPY, POMC) and others only recently discovered (e.g. AGRP, CART, OREXIN and MCH). Through direct and indirect effects on neurons expressing these neuropeptides[6], leptin maintains energy balance during starvation and energy excess. Although much has been learned about leptin action on central pathways, a great deal remains unknown, including yet unidentified genes that are transcriptionally regulated by leptin. A central feature of most cases of human and rodent obesity is "leptin resistance," defined as obesity despite high levels of circulating leptin, and resistance to the weight reducing effects of peripheral administration of recombinant leptin[7]. The mechanism of human and rodent leptin resistance is unknown and is likely, at least in part, to involve altered mRNA expression of known and unknown hypothalamic genes involved in leptin action.

Results. We have demonstrated that leptin induces suppressor-of-cytokine–signaling (SOCS)-3 mRNA in the hypothalamus of mice and rats by quantitative RT-PCR[8]. We also demonstrated that SOCS-3 is an inhibitor of leptin signal transduction. Furthermore, SOCS-3 mRNA is elevated in the hypothalamus of leptin-resistant obese Agouti mice, altogether suggesting that inappropriate elevation of SOCS-3 activity in leptin-responsive neurons may play a role in development of leptin resistance and obesity.

Future plans. We will use mRNA isolated from central and peripheral tissues of leptin-treated rodents with the goal to identify novel genes that are regulated by leptin using the GeneChip technology from Affymetrix. We will also identify differentially expressed genes in tissues from obese humans and leptin-resistant rodents.

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Cluster analysis of genome-wide expression profiles to predict gene functions with KEGG

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Although the emerging cDNA microarray technology has made it possible to observe genome-wide patterns of gene expression, there are no well-established schemes for analysing their dispersed patterns and formulating functional annota-