of the SRp40 binding site in the minigene blocked use of SSI+II. Truncation of the minigene to delete this site impaired use of SSI+II and blocked exon inclusion at early times. Transfection of muscle (L6) cells with constitutively active Akt2 mimicked insulin activation of exon 17 SS selection. Co-transfection of cells with SRp40 and the PKCβII minigene promoted 5′ SSI+II selection. Akt2 and SRp40 co-immunoprecipitated when lysates from L6 cells treated with insulin for 30 min were immunoprecipitated with SRp40 antibody followed by detection with Akt2 antibody. Further, Akt2 phosphorylated SRp40 > *in vitro*. Mutation of SRp40 Ser 81, a putative Akt site, blocked Akt2 *in vitro* phosphorylation. This work provides evidence that SRp40 is a specific substrate of Akt2 kinase that participates in the alternative splicing of PKCβII, an isozyme involved in insulin responsiveness of skeletal muscle cells. (Funded by VA Merit Review (D.R.C.) and NIDDK54393 (D.R.C.).)

126.28

The role of c-Src in protein kinase Cdelta (PKCdelta) modulation of endothelial basal barrier function

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We have previously shown that inhibition of PKCδ impairs endothelial basal barrier function. This impairment coincides with decreased FAK and RhoA GTPase activities and the disruption of stress fibers and focal contacts. To further elucidate the intracellular signaling pathway by which PKCδ modulates endothelial basal barrier function, we investigated the involvement of c-Src. Inhibition of c-Src with PP2 caused a decrease in transendothelial electrical resistance, similarly to inhibition of PKCδ with rottlerin. Transient overexpression of PKCδ did not attenuate this effect. Both rottlerin and PP2 diminished the level of active c-Src, as determined by measuring the level of tyrosine^{418} phosphorylation; however, c-Src inhibition was significantly greater in rottlerin-treated endothelial cells, as compared to PP2-treated endothelial cells. Conversely, neither inhibitor altered the level of c-Src phosphorylation at tyrosine^{529}, suggesting that Csk is not involved in rottlerin or PP2 modulation of basal barrier function. Immunofluorescence analyses demonstrated increased stress fiber formation in PP2-treated EC, while focal contacts and adherens junctions were unchanged. PP2 caused a two-fold increase in RhoA activity, however FAK activity was unchanged. These data suggest that both PKCδ and c-Src activities are necessary in maintaining competent endothelial basal barrier function. The data also suggest that PKCδ may regulate c-Src activity in endothelial cells. Thus, PKCδ may modulate endothelial basal barrier function, in part, through c-Src signaling. Funded by NIH NHLBI HL67795.

126.29

Twist, a basic helix-loop-helix transcription factor, is up-regulated in respons to androgens in prostatic cell lines

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The objective of this study was to identify androgen regulated genes expressed during development of androgen-independent prostate cancer. Using cDNA microarray technology, we identified the gene expression profile of the androgen sensitive LNCaP cell line and the LNCaP derived androgen-independent Rf and C4-2 cells. One of the identified genes was Twist. Twist is a basic helix-loop-helix transcription factor which activity has been implicated in the inhibition of differentiation of multiple cell lineages. It has also been shown to be up-regulated in respons to WNT 1, the founder member of the WNT gene family which activates the WNT pathway. Microarray analysis showed that the Twist transcript is down-regulated in the Rf and C4-2 cell lines compared to LNCaP-cells. In accordance with these results, we observed that Twist was up-regulated by androgens and DHT in LNCaP-cells and that this at least partly was a direct effect of androgens. The results were confirmed by real-time RT- PCR.

Western blot analysis of Twist was performed on various prostatic cell lines. The results showed a marked decline in Twist expression in the LNCaP-Rf and C4-2 cells when compared to the LNCaP-cells. Interestingly, in the androgen receptor (AR) negative cell line PC-3, overexpression of AR was associated with a strong induction of Twist. Analogous, silencing of AR in LNCaP cells by transfecting siRNA against AR, reduced the expression of Twist. To validate the in vivo expression of Twist, immunohistochemistry was performed on prostate tissue microarrays. Preliminary results showed decreased expression in prostate cancer tissue. Thus, we have identified Twist as a new target gene for androgens in the prostate.

Research Support; Norwegian Research Council and Mayo Foundation

126.30

Effect of neutrophil stimulation by Staphylococcus epidermidis on conditions in the phagovacuole.

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In our previous studies of the evasion of neutrophil killing by phagocytized Cryptococcus *neoformans* (*Cf*), we were able to show that survival of the organism was due to hyperacidification of the phagovacuole. This evasion was reversed, and killing accomplished when the phagovacuoles were alkalinized with chloroquin. This approach is here extended to the mechanisms of control of the phagosomal environment, and its modulation by mycobacteria, a species that evades intraphagosomal killing. Staphylococcus *epidermidis* (*Se*) is used to develop appropriate labeling methods. The fluorescent labels used (Molecular Probes, Inc.) were Oregon green™ (OG, for pH), di chlorodihydrofluorescein (DCF, for oxidative products) and DQ™ (DQ, for elastase), all covalently bound to the live bacteria; labeling did not alter their ability to stimulate neutrophils. In contrast to *Cf*, unopsonized *Se* failed to elicit any neutrophil response, as monitored by a cytoplasmic calcium spike measured with indo-1. Also, in contrast to phagovacuolar acidification seen with *Cf*, *Se* elicited a marked alkalinization. A release of oxidative products was also observed, starting approx. 1 min. after addition of the stimulus, a time period in accord with our previous kinetic studies of neutrophil stimulus responses. While there was concomitant release of elastase into the phagovacuole it could not be quantitated because Se alone appears to release that enzyme at 37o. All of our measurements were made by kinetic flow cytometry in order to make cell-by-cell observations and to avoid measuring effects of neutrophil-released products on residual stimuli in solution. Research supported by NIH DK31056 and HL76463.

126.31

Essential role of Nrf2 in protection against ovarian follicle loss induced by 4vinylcyclohexene and 4-vinylcyclohexene diepoxide in mice.

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4-Vinylcyclohexene (VCH) and its metabolite 4-vinylcyclohexene diepoxide (VCD) represent a potential health hazard, because they selectively destroy oocytes in small pre-antral follicles leading to premature ovarian failure in animals. Previous studies suggest that metabolism of VCH and VCD by phase I and II drug-metabolism enzymes plays an important role in the ovotoxicity of these chemicals. Nrf2 is a member of the Cap "N" Colar bZip family of transcription factors that mediates the basal expression and induction of phase II enzymes such as NQO1 and GST. In this study, we examined the role of Nrf2-regulated gene expression in the ovotoxicity of VCH and VCD by using Nrf2 knockout mice. Immature (age, day 28) female wild-type and Nrf2-/- mice (both in B6 background) were treated with VCH or VCD using established protocols; 4 h following the final dose, ovaries were collected. Complete serial sections of ovaries were evaluated histologically for the presence of follicles. As expected, the primordial and primary follicle numbers in ovaries from wild type mice decreased significantly ($p<0.05$) following treatment with either VCH or VCD. However, the primordial and primary follicles in ovaries from Nrf2-/- mice exhibit much higher sensitive to the toxicity of VCH and VCD than those of wild type mice. Currently, the phase II enzyme related mechanism is being investigated in ovaries with immuno-histo-chemistry and other approaches. Taken together, the findings suggest that loss of Nrf2 function and Nrf2-mediated expression of phase II

genes may play an important role in detoxification of VCH and VCD, thereby protecting ovarian follicles from the ovotoxicity of the chemicals. This study is supported by NIOSH/HELD/CDC.

126.32

Regulation of a protein phosphatase-1I complex in global cerebral ischemia

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The catalytic subunit of protein phosphatase-1 ($PP\text{-}1_C$) interacts with BAD, Rb and other proteins that are involved in the control of cell death and that become dephosphorylated upon activation of cell death pathways. The major form of protein phosphatase-1 in brain is protein phosphatase-1_I ($PP\text{-}1_I$), a Mg^{2+}/ATP-dependent form of PP-1 that contains $PP\text{-}1_C$ and the inhibitor-2 regulatory subunit. We hypothesize that $PP\text{-}1_I$ is an important component of the cell signaling pathways that control the induction of cell death in global cerebral ischemia. Four forms of $PP\text{-}1_I$ have been identified, purified and characterized from pig brain: $PP\text{-}1_{IA}$, $PP\text{-}1_{IB}$, $PP\text{-}1_{IC}$ and $PP\text{-}1_{ID}$. Each form has a distinct quarternary structure (molecular mass by gel filtration = 140 kDa, 140 kDa, 250 kDa, and 250 kDa, respectively). All 4 forms of $PP\text{-}1_I$ contain $PP\text{-}1_C$, inhibitor-2, one or more activating kinase(s), and a distinct pattern of other proteins identified by SDS/PAGE that may represent novel regulatory subunits and substrates; these proteins are currently being analyzed by mass spectrometry. $PP\text{-}1_{IC}$ activity increased up to 5-fold during global cerebral ischemia following complete cardiac arrest. The properties and subunit composition of the $PP\text{-}1_{IC}$ complex purified from non-ischemic control brain are quite distinct from those of the $PP\text{-}1_{IC}$ complex purified from brain subjected to global ischemia. These results suggest that $PP\text{-}1_{IC}$ is an important component of the cell signaling pathways activated by global cerebral ischemia, and thus may control the cellular and molecular responses to ischemiain brain. Modulation of $PP\text{-}1_{IC}$ or its activating kinase(s) represents a potential therapeutic target in global cerebral ischemia.

126.33

Regulation of the renal vitamin D 1alpha-hydroxylase cytochrome P450 (CYP27B1) and ferredoxin by dietary phosphorus in young and adult rats

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Young rats adapt to a low phosphorus diet by increasing plasma levels of 1,25-dihydroxyvitamin D (1,25D), the biologically active metabolite of vitamin D. 1,25D then increases intestinal phosphate absorption. Previous studies have shown that the capacity of rats to adapt to low dietary phosphorus declines with age. The purpose of this study was to determine whether this decreased adaptation was due to decreased expression of the renal 1alpha-hydroxylase (1-OHase), which makes 1,25D. The 1-OHase consists of ferredoxin reductase, ferredoxin, and a terminal cytochrome P450 – CYP27B1. Young (2 months) and adult (12 months) F344 rats were placed on a low phosphorus (0.1%) or high phosphorus (1.0%) diet for 2 weeks. Plasma 1,25D was markedly increased by the low phosphorus diet in young animals but not in adults. To determine whether this difference was due to decreased 1-OHase expression, mRNA levels of CYP27B1 and ferredoxin were measured by ribonuclease protection assay. In young animals, the low phosphorus diet increased renal CYP27B1 mRNA levels 8-fold compared to the high phosphorus diet, but there was no significant effect in adults. Regarding ferredoxin mRNA levels, there was no effect of dietary phosphorus in either age group. The effect of low dietary phosphorus on plasma calcium, phosphate, and parathyroid hormone levels was similar in both age groups. These results suggest that the decreased CYP27B1 expression in the adult in response to dietary phosphorus depravation is not due to changes in the major regulators of 1-OHase activity.

126.34

Plant defensin-resistant yeast mutants with increased life-span

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Plant defensins are small, basic cysteine-rich peptides that inhibit the growth of a broad spectrum of yeast and fungal species, including *Candida albicans*. We demonstrated that plant defensins induce membrane permeabilization in susceptible fungi after a specific interaction with binding sites on the fungal plasma membrane, which are composed of complex sphingolipids. Yeast mutants affected in the biosynthesis of such sphingolipids are resistant to certain plant defensins, but do not display resistance to other stress agents such as paraquat, a redoxactive component that mediates its toxic effect via the production of reactive oxygen species. Conversely, yeast mutants characterized by paraquat-resistance, such as *sch9*-deletion mutant, remain as sensitive towards plant defensins as the corresponding wild-type strain confirming that resistance to paraquat and plant defensins are normally uncoupled.

Recently, however, we isolated a *Saccharomyces cerevisiae* transposon mutant (ScTnDm24d) which is 20-fold more resistant to different plant defensins as well as to paraquat, when compared to the corresponding wild-type strain. Moreover, this mutant is also resistant towards other stresses, such as nutritional stress, and it displays a 10-fold extension of the mean chronological life-span, which is probably the highest life-span extension reported so far. At present, we are determining the exact localisation of the transposon insert as a start to explain in more detail why ScTnDm24d shows the phenotypic characteristics described.

This research was supported by grants of FWO Vlaanderen, IWT Belgium and the Research Council of the K.U. Leuven.

126.35

***In vivo* bypass efficiencies and mutational signatures of the guanine oxidation products 2-aminoimidazolone and 5-guanidino-4-nitroimidazole**

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DNA oxidation is implicated as a cause of aging and age-related diseases. As a model of age-related guanine oxidation mutagenesis, wild-type AB1157 *E. coli* cells were transformed with single-stranded M13mp7L2 bacteriophage genomes site-specifically containing 2-aminoimidazolone (Iz) or 5-guanidino-4-nitroimidazole (NI). Progeny phage were isolated and used to determine the *in vivo* DNA polymerase bypass efficiencies and mutation frequencies of the DNA lesions. Iz was efficiently bypassed and 88% mutagenic, inducing almost exclusively G→C mutations. In contrast, NI was a strong replication block and 55% mutagenic, generating G→A, G→T, and to a lesser extent, G→C mutations. SOS-induction did not change the bypass efficiency of Iz significantly, but increased the bypass efficiency of NI nearly 10-fold. The mutation frequencies of both lesions decreased in SOS-induced cells. Our results suggest these lesions are substrates for one or more of the SOS-induced lesion bypass polymerases and demonstrate that Iz and NI are potent contributors of point mutations *in vivo*.

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126.36

Pesticides and Neurodegeneration

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Several studies indicate that exposure to certain pesticides may cause degeneration of the nigrostriatal pathway leading to changes in dopamine levels and its metabolites and production of increased levels

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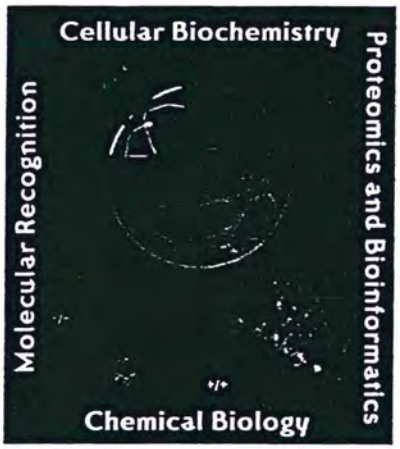

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ABSTRACTS

Abstracts 1.1–147.3
Indexes

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