1. INTRODUCTION

1.1 Regulation of gene expression by oxygen

The ability of an organism to adapt to changes in the oxygen concentration is essential for its survival. Accordingly, the expression of numerous genes, products of which are involved in various processes such as hematopoesis, fibrinolysis or carbohydrate metabolism is regulated by oxygen (Tab. 1).

Under physiological conditions the mean oxygen tension in arterial blood is maintained at levels of 74-104 mm Hg (normoxia) and in venous blood at 34-46 mm Hg (mild hypoxia). Oxygen tensions higher than physiological (hyperoxia) could lead to cellular damage due to production of reactive oxygen species (ROS) while oxygen tensions lower than physiological (hypoxia) is dangerous because oxygen is necessary for the functioning of the mitochondrial electron transfer chain and many other metabolic pathways.

It is believed that the general scheme of the oxygen-dependent gene regulation in the case of either hypoxia or normoxia in mammals is similar (Fig. 1). The first stage in this process is oxygen sensing by a sensor molecule that could alter its conformation and/or enzymatic activity under different oxygen concentrations. The next step is signal transduction due to the production of mediators or second messengers which modify regulatory molecules such as DNA-binding transcription factors. These oxygen-modulated transcription factors, which may be different for hypoxic and normoxic conditions, bind in the final step specific response elements in the promoters of oxygen-regulated genes.

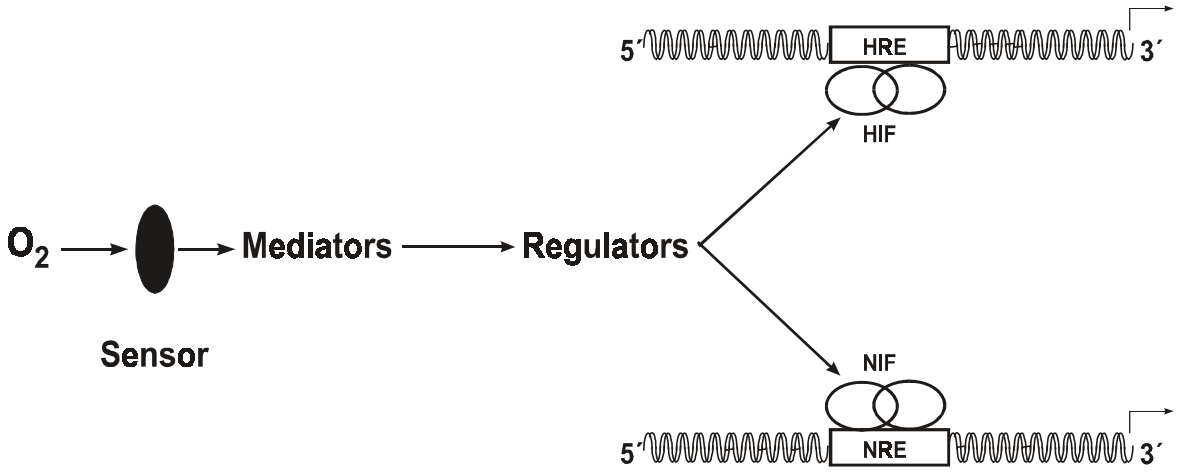

Fig. 1: **Model of the oxygen signalling pathway regulating gene expression.** See text for explanation. HRE, hypoxia response element; NRE, normoxia response element; HIF, hypoxia-inducible factor; NIF, normoxia-inducible factor

Tab. 1: **O_2-modulated processes and genes in mammals.**

Processes	Hypoxia-induced gene expression	Normoxia-induced gene expression
Hematopoesis	Erythropoietin (EPO)[1]	
Angiogenesis	Vascular endothelial growth factor (VEGF)[2]	Placental growth factor (PLGF)[3]
Fibrinolysis	Plasminogen activator inhibitor-1 (PAI-1)[4]	Plasminogen activators [5]
Wound healing/ Inflammation	Inducible nitric oxide synthase (iNOS)[6]	Tumor necrosis factor-α (TNF-α)[7]
Development/ Differentiation	p53 Tumor supressor protein[8]	
	bcl-2 Apoptosis preventing protein[9]	
	Insulin like growth factor binding protein-1 (IGFBP-1)[10]	
Antioxidant defense		Glutathione peroxidase[11]
		Catalase[12]
		Superoxid dismutases[13]
Amino acid metabolism		Tyrosine aminotransferase[14]
Glykolysis/ Gluconeogenesis	Glucokinase (GK)[15]	Cytosolic phosphoenol- pyruvate carboxykinase-1 (PCK-1)[16]
	Aldolase A (ALD-A)[17]	
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)[18]	
	Phosphoglycerate kinase-1 (PGK-1)[19]	
	Pyruvate kinase L (PK_L)[20]	
	Pyruvate kinase M (PK_M)[21]	
	Lactate dehydrogenase A (LDH-A)[22]	

(1) (Jiang et al., 1996); (2) (Forsythe et al., 1996; Levy et al., 1995; Liu et al., 1995); (3) (Gleadle et al., 1995); (4) (Kietzmann et al., 1999; Samoylenko et al., 2001; Kietzmann et al., 2003b); (5) (Pinsky et al., 1998); (6) (Melillo et al., 1995); (7) (Wibbenmeyer et al., 1995); (8) (Koumenis et al., 2001); (9) (Tamatani et al., 1998); (10) (Tazuke et al., 1998); (11); (12) (Li et al., 1989); (13) (Jackson et al., 1996); (14) (Nauck et al., 1981); (15) (Kietzmann et al., 1997); (16) (Hellkamp et al., 1991); (17); (18) (Graven et al., 1999); (19) (Firth et al., 1994); (Semenza et al., 1996); (20) (Krones et al., 2001); (21) (Iyer et al., 1998); (22) (Firth et al., 1995).

1.1.1 Oxygen gradients and metabolic zonation of the liver

Oxygen concentration gradients are formed due to differences in the blood supply within some organs and tissues. Such gradients exist physiologically in organs such as liver and to some extend in kidney, during fetal development and pathophysiologically during tumor formation.

In the liver, the smallest functional unit is represented by the acinus. Within the acinus the blood flows from the region around the hepatic artery and terminal portal vein (periportal zone) into the sinusoids towards the cells located in the region around the central vein (perivenous zone) (Jungermann et al., 1996b; Jungermann et al., 2000). Due to the metabolism of the cells within the sinusoid an oxygen gradient is formed reaching from about 60-65 mm Hg in the periportal area to about 30-35 mm Hg in the perivenous zone. Furthermore, the expression of most genes for enzymes, translocators and receptors is different between the cells of the two zones (Fig. 2). Many genes are expressed predominantly in the first, periportal, or in the second, perivenous, half of the acinus while some genes are expressed or inhibited within only the first or the last quarter of the acinus. The expression of the genes for the rate-controlling enzymes of gluconeogenesis, phosphoenolpyruvate carboxykinase-1 (PCK-1) and fructose-1,6-bisphosphatase

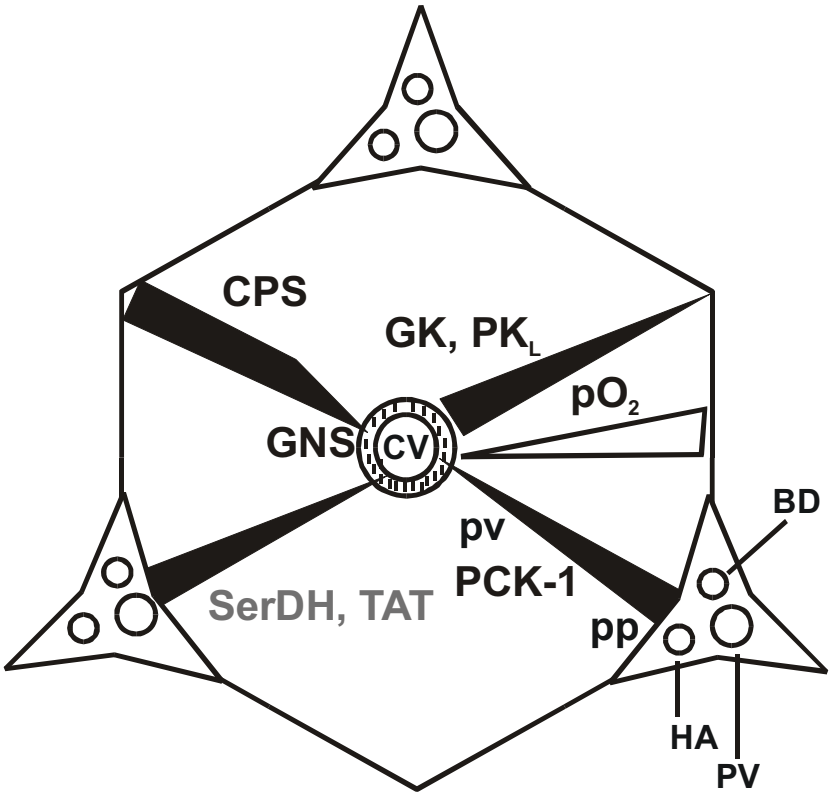

Fig. 2: **Zonation of gene expression in liver.** Scheme of the liver acinus and existing gradients in gene expression as well as of the oxygen tension (pO_2). BD, bile duct; CPS, carbamoylphosphate synthetase; CV, central vein; GK, glucokinase; GNS, glutamine synthetase; HA, hepatic arteriole; PK_L, pyruvate kinase liver type; pp, periportal; PV, portal venule; pv, perivenous; SerDH, serine dehydratase; TAT, tyrosine aminotransferase. Arrows give the gradients in expression.

(FBPase), as well as for the amino acid metabolizing enzymes serine dehydratase (SerDH) and tyrosine aminotransferase (TAT), which are related to gluconeogenesis, are expressed predominantly in the periportal zone, as has been shown for the mRNA, proteins and enzyme activities (Jungermann et al., 1989; Bartels et al., 1990; Ogawa et al., 1994; Ogawa et al., 1995). Furthermore, the key ureagenic enzyme carbamoylphosphate synthetase (CPS) is also expressed periportally (Moorman et al., 1988). In contrast, the genes for the rate-controlling glycolytic enzymes, glucokinase (GK) and pyruvate kinase type L (PK_L), are expressed mostly in the perivenous zone (Jungermann et al., 1989). The mRNA and protein for glutamine synthetase (GNS) were detected exclusively in parenchymal cells of the distal perivenous area (Fig. 2) (Gebhardt et al., 1988; Moorman et al., 1988). These zonal differences in the gene expression pattern constitute the basis for the zonal differerent metabolic capacities. Thus, the capacity for oxidative energy metabolism, glucose output, protective metabolism, urea, cholesterol and bile formation is greater in the periportal area, whereas the capacity for glucose uptake, glutamine formation and xenobiotic metabolism is higher in the perivenous area. Due to the fact that all hepatocytes possess the same genome it was considered that concentration gradients of hormones, metabolic substrates, products and oxygen which are formed during the passage of blood through the sinusoid may be determinants for the zonated gene expression. In line with this, it was shown that the periportal to perivenous gradient in oxygen tension appears to be a key regulator for expression of the periportal PCK and perivenous GK in liver.

1.1.2 Regulation of zonated gene expression by normoxia

The genes such as PCK-1 which are mainly expressed under periportal or normoxic conditions could be either specifically inhibited by low oxygen tensions or induced by a normoxia-induced transcription factor or factors. In addition to PCK-1, other genes induced by normoxia have been identified (Tab. 1), among these are placental growth factor (PLGF) (Gleadle et al., 1995), tissue-type and urokinase-type plasminogen activators (tPA and uPA) (Pinsky et al., 1998), atrial natriuretic peptide clearance receptor (ANP-CR) (Sun et al., 2000), and antioxidant enzymes such as glutathione peroxidase (Li et al., 1989), catalase (Li et al., 1989), manganese-containing and copper/zinc-containing superoxide dismutase (Jackson et al., 1996). In particular, normoxia-induced expression was demonstrated for a number of genes which are predominantly expressed in the periportal region of the liver acinus, such as the heme-binding protein hemopexin (Kietzmann et al., 1995), gluconeogenic enzymes phosphoenolpyruvate carboxykinase-1 (PCK-1) (Hellkamp et al., 1991) and tyrosine aminotransferase (TAT) (Nauck et al., 1981). However, the mechanisms, regulatory transcription factors and the DNA

responsive elements required for normoxia-dependent gene regulation are not completely known and a general view has not been reached.

Many models for oxygen-dependent gene regulation involve signal transduction via oxygen-binding hemproteins and generation of reactive oxygen species (ROS) as mediators. The role of a heme-containing protein as oxygen sensor is based on experiments in which carbon monoxide (CO) which stabilizes the oxy conformation of heme as well as heme synthesis inhibitors such as succinylacetone prevented the response to hypoxia. By contrast, cobalt and nickel, which are incorporated into heme, locked its deoxy state thus mimicking hypoxia (Goldberg et al., 1988; Kietzmann et al., 1997; Jungermann et al., 1997). Spectrophotometric experiments with carotid body preparations and HepG2 cells showed that heme-containing b-type cytochromes such as the NAD(P)H oxidase (Gorlach et al., 1993; Fandrey et al., 1994a) may have a role in the oxygen signalling pathway. By contrast, other experiments with cardiomyocytes pointed also to a role of cytochrome c oxidase in oxygen sensing (Wilson et al., 1994).

It was shown that production of intracellular ROS such as H_2O_2 was increased by high and decreased by low oxygen tension (Kietzmann et al., 1997). Chelators of iron such as desferrioxamine can mimic hypoxia (Wang et al., 1993; Gleadle et al., 1995) which could be explained by the assumption that hydroxyl anions and hydroxyl radicals, produced in the presence of Fe^{2+} from H_2O_2 in a Fenton reaction, were also involved in the transduction of the oxygen signal. This was corroborated for the PCK-1 gene since H_2O_2 mimicked the action of periportal pO_2 (Kietzmann et al., 1996) and two Fenton reaction inhibitors, the iron chelator desferrioxamine (DSF) and the hydroxyl radical scavenger dimethylthiourea (DMTU) (Kietzmann et al., 1998) mimicked perivenous pO_2.

Until now, a common normoxia responsive DNA element in the promoters of normoxia-regulated genes has not been identified. Two oxygen-responsive elements (ORE-1 and ORE-2), which shared the consensus sequence 5'-AYCCTCYRAGAAA-3' (Y=A or T; R=A or G), have been identified in the 5'-flanking region of the human glutathione peroxidase (GPX) gene (Cowan et al., 1993).

In experiments with primary rat hepatocytes, a normoxia response element (NRE) 5'-TTAGGTCAG-3' was located in the PCK-1 promoter at –146/-138 (Bratke et al., 1999) and shown to be involved in the positive modulation by oxygen of the glucagon-dependent PCK-1 expression (Bratke et al., 1999). Although the respective transcription factor binding to the NRE is unknown yet, it might be possible that similar elements and factors may also account for the periportal expression of other genes.