ras mutations are known to be a common event in tumorigenesis. Although the various RAS isoforms differ only for the last 24 aminoacids and all of them appear to be involved in the same pathways of signal transduction, in colorectal carcinomas (CRCs) the mutations affect almost exclusively the K-*ras* gene, while H-*ras* mutations are mostly found in bladder carcinomas and N-*ras* mutations in leukaemia cells. In almost all cases, the genetic alteration is a point mutation in codons 12 or 13, and less frequently in codon 61. By affecting the GTPase activity of the protein, they always lead to a constitutively active protein. However, recent data, obtained in different experimental systems or by analysis of primary and metastatic tumors, suggest that mutations in different codons, different mutations in the same codon and mutations of different isoforms of RAS may have diverse biological consequences. In particular, our previous analysis of 160 primary CRCs indicated that codon 12 K-*ras* mutations may have a role in the mucinous differentiation pathway of these tumors, while codon 13 mutations are associated to more aggressive carcinomas. The purpose of our present work is to evaluate whether the effects of the expression, in a same cell type, of K-*ras* mutated in codon 12 or in codon 13 are indeed different. We also intend to establish whether the same type of mutation in codon 12 of K-*ras* and H-*ras* differentially affects the cells.

The studies are performed on HT-29, a human colorectal adenocarcinoma cell line in which the endogenous *ras* genes are wild type. We have obtained stable clones of HT-29 transfected with the pSwitch vector and the companion mifepristone-inducible eukaryotic expression vector pGene/V5-HisB (Gene Switch ™ System, Invitrogen) containing the K-*ras* cDNA mutated in codon 12 (K-RAS G12V) or in codon 13 (K-RAS G13D) and the H-*ras* cDNA mutated in codon 12 (H-RAS G12V). The expression of each of these cDNAs differentially affects the cells in terms of proliferative activity and cellular morphology; the effects on gene expression are currently under investigation.

20. AMINO ACID BIOSYNTHESIS IN *STREPTOMYCES COELICOLOR*

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Purpose of the study: Amino acids are key precursors for the biosynthesis of many antibiotics. In *Streptomyces coelicolor* both L- and D-tryptophan are incorporated in calcium dependent antibiotic (CDA), and products of catabolism of branched chain aliphatic amino acids are precursors of polyketide biosynthesis.

Unlike the well known and tight regulation of amino acids biosynthesis in enteric bacteria little is known in *Streptomyces*.

As a physiological link between tryptophan and histidine biosynthesis is observed (*S. coelicolor his*A gene is involved both in histidine and tryptophan biosynthesis) we also carried out investigations on the histidine biosynthesis pathway.

Summary of the methods: Tryptophan biosynthetic gene expression has been investigated through Real Time RT-PCR at different growth stages in *Streptomyces coelicolor*.

Results and conclusion: the *trp* genes analysed, including those localized in the CDA cluster, appear to be regulated by growth phase but not by feedback repression. Only two genes, *trp*E1 and *trp*E3, seem to be repressed by the end product. Regarding the histidine biosynthetic pathway, we found that two different Orfs, *SCO5208*, a putative monophosphatase, and *SCO2771*, a hypothetical protein, complement the mutation of a *S. coelicolor* strain deficient in HolPase activity. Moreover, *SCO2771*, cloned in high copy number, causes an early production of the polyketide antibiotic actinorhodin.

21. CADMIUM AS AN APOPTOTIC INDUCER IN SEA URCHIN EMBRYOS: POSSIBLE IMPLICATION OF AN EXTRINSIC PATHWAY

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Cadmium is a heavy metal toxic to living organisms even at low environmental concentrations. Under physiological conditions it does not exert any biological role, it is not degraded by microorganisms, and once it is assimilated in the cells, it cannot be removed and accumulates during time. The toxicity of cadmium and the mechanisms through which it acts are still under study, however some of the effects produced at cell level are known. In the majority of cases it damages chromosomes and inhibits the mechanisms implicated in DNA repair. Numerous researchers demonstrated that heavy metals inhibit embryogenesis and interrupt development at different stages.

In the present work, we studied the effects of continuous exposure to cytotoxic concentrations on a model system, *Paracentrotus lividus* embryos. We demonstrate that continuous exposure of sea urchin embryos to 1 mM $CdCl_2$ induces synthesis of specific stress proteins when the exposure time is < 21h (Roccheri et al., 2004, BBRC 321: 80).

Using AAS experiments, we assayed the bioaccumulation of cadmium in sea urchin embryos treated since fertilization with 1 mM $CdCl_2$. An intracellular increase in the amount of this metal was observed during time. TUNEL assays on whole mount embryos showed that a long exposure triggers severe fragmentation of DNA. Immunocytochemical experiments on embryos treated with cadmium and reacted with anti-cleaved-caspase-3 and anti-pro-caspase-3 antibodies, showed an increase of cleaved caspase-3 and a decrease of pro-caspase-3, depending on the length of treatment. Likewise the activation of cleaved forms of α-Fodrin (an anchorage cytoskeleton protein) and Lamin A (nuclear membrane protein), substrates of caspase-3, has been shown to increase as the exposure time to cadmium increased. Using since fertilization the caspase inhibitor Z-DEVD FMK, which specifically binds caspase-3, we have demonstrated, by immunocytochemical experiments with anti-caspase-3, anti-α-Fodrin and anti-Lamin A antibodies, that these proteins result inactive.

Therefore we followed the role of caspase-7 in the cadmium-induced apoptotic pathways. Our results showed that also caspase-7 is involved in this mechanism. The possible implication and regulation of intrinsic and extrinsic apoptotic pathways has to be investigated. We have just studied the expression of an initiator enzyme implicated in the extrinsic pathway, the caspase-8. The activation of this caspase has resulted to be time dependent during cadmium treatment. Instead, caspase-10 activation showed a different progress: caspase-10 is actively cleaved during physiological apoptosis, while it is not involved in apoptosis induced by cadmium.

These data suggest that an extrinsic apoptotic pathway could be activated under stress by cadmium, and the starter caspase should be caspase-8 and not caspase-10. There is no evidence in literature that the activation of the apoptotic extrinsic pathway is triggered by cadmium, so it will be necessary to explain how cadmium can cause the activation of this pathway in sea urchin embryo.

22. PROTEOME ANALYSIS OF THE ANTIBIOTIC PRODUCER *AMYCOLATOPSIS BALHIMYCINA*

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Glycopeptides are an important class of antibiotics with vancomycin and teicoplanin currently in clinical use. They are often "drugs of the last resort" in treating life-threatening infections but the emergence of resistance to glycopeptides among enterococci (the so called VRE strains, for vancomycin-resistant enterococci) and the fear that this high level resistance may eventually become widespread in methicillin-resistant *Staphylococcus aureus* (MRSA) has renewed the interest in this class of antibiotics.

The actinomycete, *Amycolatopsis balhimycina,* produces the glycopeptide vancomycin-like balhimycin. Its full genome sequence is in progress within the European COMBIG-TOP project, while the *bal* cluster and the biosynthetic pathway responsible for balhimycin biosynthesis have been extensively studied. Up to day, little is known about the relationships between primary metabolism and antibiotic production.

A proteome study, based on 2-D gel electrophoresis and MS-MALDI-TOF analysis, was performed to create a proteome reference 2D-map and to analyse the global protein expression profile before and during balhimycin production (i.e. after 18 and 42 h of growth, respectively) and between the wild type (wt) and the non producing SP1 mutant strain (Pelzer *et al*., 1999) at late growth stages (i.e. 42 h of growth).

134 proteins were identified and used to create the reference 2D-map. This study confirmed the presence of proteins previously annotated as hypothetical