54° CONVEGNO GRUPPO EMBRIOLOGICO ITALIANO

4-7 giugno 2008 ROMA

In onore di Alberto Stefanelli

Dipartimento di Biologia Animale e dell'Uomo: Roma "La Sapienza"

Dipartimenti di Biologia: Roma Tor Vergata e Roma 3

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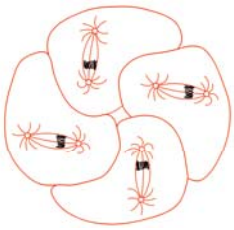
UNIVERSITA' degli STUDI di ROMA TOR VERGATA

SAPIENZA UNIVERSITÀ DI ROMA

Segreteria
e-mail convegno.gei@gmail.com

54° CONVEGNO DEL GRUPPO EMBRIOLOGICO ITALIANO
Roma 4-7 Giugno 2008



PROGRAMMA

Mercoledì 4 giugno
(Dipartimento di Biologia Animale e dell'Uomo - Anatomia Comparata - Via Borelli 50)

10.00 **Apertura della Segreteria: Registrazioni**

16.00-16.30 ***Benvenuto ed introduzione di E. Manelli. Saluti delle Autorità***

Simposio neurobiologia
Moderatore: E. Manelli

16.30-17.00 *Capanna E. (Università Roma La Sapienza)*
Non solo Mauthner: 70 anni di ricerche di neurobiologia di Alberto Stefanelli

17.00-17.30 *Zottoli J. S., Cioni C. Seyfarth E.A. (Università Roma La Sapienza)*
I neuroni reticolospinali degli anamni: celebrazione del contributo di Alberto Stefanelli alle neuroscienze comparate

17.30-18.15 ***Cocktail di benvenuto***

18.15-18.45 *Augusti-Tocco G. (Università Roma La Sapienza)*
Potenzialità di precursori neurali derivati da diverse regioni del sistema nervoso

18.45-19.15 *Giacobini P. , Fasolo A. (Università Torino)*
Il lungo viaggio dei neuroni GnRH-1

Giovedì 5 Giugno
(Dipartimento di Biologia Animale e dell'Uomo - Anatomia Comparata - Via Borelli 50)

8.45-9.00 Ricordo dei Proff. E. Ghirardelli, V.G. Leone, E. Parisi

Simposio cellule staminali
Moderatori: G. Barsacchi, M. Pestarino

9.00-9.30 *Cossu G. (Università Milano e Istituto San Raffaele)*
Origine, potenzialità e destino differenziativo dei mesoangioblasti,

progenitori mesodermici della parete vascolare

9.30-10.00 *Mangia F. Università Roma La Sapienza*
The oncogenic factor TCL1 regulates the blastomere proliferation of early mouse preimplantation embryos by interacting with the AKT2 isoform of protein-kinase PKB/AKT

10.00-10.15 *Conte M., Deri P., Mannini L., Batistoni R. (Università Pisa)*
L'espressione di *Djmot*, un membro della famiglia multigenica di proteine HSP70, è necessaria per la proliferazione delle cellule staminali delle planarie

10.15-10.30 *Casarosa S., Vitobello A., Lei L., Andreazzoli M., Cremisi F., Vignali R., Barsacchi G. (Università Pisa)*
Come trasformare cellule staminali embrionali in cellule retiniche

10.30-10.45 *Candiani S., Marcoli M., Tonachini L., Monticane M., Mastrogiacomo M., Ottonello A., Cervetto C., Palazzi P., Maura G., Cancedda R., Castagnola P., Pestarino M. (Università Genova)*
Gamma amino butyric acid (GABA) receptors in induced neuron-like Bone Marrow Stromal Cells

10.45-11.15 ***pausa caffè***

Sviluppo e differenziamento del sistema nervoso
Moderatori: G. Augusti Tocco e I. Nardi

11.15-11.30 *Manni L., Zaniolo G., Burighel P. (Università Padova)*
Sviluppo e regressione del sistema nervoso in *Botryllus schlosseri* (Ascidiacea)

11.30-11.45 *Vizioli J, Croq F., Lefebvre C., Salzet M., Pestel J., Sautière P.E.. (Università Lille – Francia)*
Caratterizzazione della progranulina nel sistema nervoso centrale della sanguisuga *Hirudo medicinalis*

11.45-12.00 *Tata AM., Loreti S., De Angelis F., Bernardo A., Magnaghi V., Minghetti L., Augusti Tocco G. (Università Roma La Sapienza)*
Effetti opposti dell'Acetilcolina sulla proliferazione e il differenziamento di cellule gliali mielinizzanti

12.00-12.15 *Nardacci R., Perfettini J.L., Amendola A., Kroemer G. Piacentini M. IRCCS Lazzaro Spallanzani – Roma)*
Meccanismo molecolare di morte cellulare programmata in cellule multinucleate aberranti

12.15-12.30 *Aluigi M.G., Coradeghini R., Guida C., Scanarotti C., Bassi A.M., Falugi C., Tagliafierro G., Ferrando S., Santi P., Raposio E.(Università Genova)*
Pre-adipocytes: commitment to cholinergic neurogenesis

12.30-12.45 *Ori M., Reisoli E., De Lucchini S., Nardi I. (Università Pisa)*
Ruolo del recettore serotoninergico 5-HT2B nella retinogenesi e nella morfogenesi craniofacciale durante lo sviluppo di *Xenopus laevis*

12.45 -14.30 ***pausa pranzo***

14.30-14.45 *Giudetti G., Giannaccini M., Biasci D., Capriotti C., Andreazzoli M., Barsacchi G.(Università Pisa)*
Geni regolati dal fattore di trascrizione *Xrx1*: analisi per mezzo di microarray

14.45-15.00 *Mancini P., Barsacchi G., Galli A., Vignali R. (Università Pisa)*
Alla ricerca di interattori molecolari dei fattori di trascrizione XOTX2 e XOTX5b

15-15.15 *Cappelletti G., Ronchi C.,Cartelli D., Tedeschi G. ,Nonnis S., Toscano A.,Giavini E. (Università Milano)*
Nitrazione di proteine: quale ruolo nel differenziamento neuronale

15.15-15.30 *Ferrando S., Bottaro M., Ferrando T, Gambardella C.,Tagliaferro G. (Università Genova)*
Differenziamento dell'epitelio olfattivo nell'elasmobranco *Raya clavata*

15.30-15.45 *Avallone B, Sasso F., Balsamo G., Marmo F. (Università Napoli "Federico II")*
Il salicilato attenua l'ototossicità della gentamicina nella papilla basilaris della lucertola *Podarcis sicula*

15.45-16.00 *Moreno S., Cimini A., Cristiano L., Giardi F., D'Angelo B., Carrara P., D'Amelio M., Cerù M.P. (Università Roma Tre)*
I perossisomi durante l'invecchiamento cerebrale. Studio morfologico e biochimico nella neocorteccia e nell'ippocampo di topo

16.00-16.15 *Battaglia P.A., Gigliani F.*
Lo stato attuale della ricerca delle basi neuronali e genetiche della rappresentazione simbolica

16.15-16.45 ***pausa caffè***

Aspetti della gametogenesi e dello sviluppo
Moderatori: C. Taddei e P. Andreuccetti

16.45-17.00 *Vaccaro M., Graziani F., Gigliotti S., Carotenuto R., Campanella C. (Università Napoli "Federico II")*
XNOA 36 and spectrin mRNAs are located in half of Xenopus oocyte bisecting the mitochondrial cloud

17.00-17.15 *Ballarin L., Menin A. (Università Padova)*
In bilico tra vita e morte: il ciclo blastogenetico coloniale dell'ascidia *Botryllus schlosseri*

17.15-17.30 *Viscuso R., Brundo M.V., Sottile L. (Università Catania)*

Attività spermiofagica nelle vie genitali maschili di Orthoptera Acrididae e Tettigoniidae

17,30-17.45 *Pinsino A.,Agnello M.,Filosto S.,Bosco L. Roccheri M.C. (Università Palermo)*
Effetti del cadmio e del manganese sullo sviluppo embrionale del riccio di mare

17.45-18.00 *Carra E., Salerno B., Gerardi E., Rinaldi A.M. (Università Palermo)*
Astenozoospermia e DNA mitocondriale

18.00-18.15 *Prisco M.,Valiante S., Apicella E., Agnese M., Del Giudice G., Angelini F., Laforgia V., Andreuccetti P. (Università Napoli "Federico II")*
The expression of PACAP and PACAP receptors during spermatogenesis of cartilaginous fish *Torpedo marmorata*

18.15-18.30 *Maurizii M.G. Cavaliere V., Gamberi C., Lasko P. Gargiulo G., Taddei C., (Università Bologna)*
Analysis of the distribution of Vasa protein during the early oogenesis of the lizard *Podarcis sicula*

21.00 **Cena sociale, Ristorante "La Mimosa", Via Bari 11a**

Venerdì 6 Giugno
(Dipartimento di Biologia- Università ROMA TRE, Viale Marconi 446)

8.00 Servizio navetta da Globus Hotel a Viale Marconi 446

Simposio biologia dello sviluppo dei vegetali
Moderatore: M. Grilli Caiola

9.15 – 9.30 *Introduzione al Simposio: M. Grilli Caiola (Università Roma Tor Vergata)*

9.30 -10.00 *Savona M. , Falasca G., Ruffoni B., Trovato M. , De Vries S., Altamura M. M. (Università Roma La Sapienza)*
Studio dell'induzione dell'embriogenesi somatica in ciclamino (*Cyclamen persicum* Mill.)

10.00-10.30 *Berta G. Gamalero E., Lingua G., Fusconi A. (Università Piemonte Orientale)*
Effetti di microrganismi rizosferici sullo sviluppo radicale delle piante, in sistemi sperimentali diversi

10.30 -11.00 *Bitonti M.B., Bruno L., Chiappetta A., Iaria D., Baraldi R., Van Onckelen H., Fambrini M., Michelotti V., Salvini M., Pugliesi C. (Università Della Calabria)*
Basi molecolari e citofisiologiche dell'epifillia manifestata da un variante somaclonale dell' ibrido interspecifico *Helianthus annuus L. x H. tuberosus*

11.00 -11.15 *Serafini-Fracassini D.*, *Della Mea M., Iorio R. e Del Duca S (Università Bologna)*
La transglutaminasi, un enzima visto nelle sue attività nelle piante e negli animali

11.15 -11.30 *Tisi A., Angelini R., Moreno S., Lucretti S., Federico R. Cona A.(Università Roma Tre)*
La spermidina inibisce la progressione del ciclo cellulare ed induce morte cellulare nella radice primaria di plantule di mais

11.30 - 12.00 **pausa caffè**

Regolazione genica e differenziamento
Moderatori: De Bernardi F. e Giavini E.

12.00-12.15 *Conte D., Arnone S.,Ceccarelli A (Università Torino)*
Regolazione differenziale dell'espressione di Dpc3 durante crescita e differenziamento: identificazione di una nuova popolazione di Anterior-Like Cells in *D. discoideum*

12.15-12.30 *Candiani S., Moronti L., Pestarino M. (Università Genova)*
Studio del locus colinergico nell'anfiosso *Branchiostoma floridae*

12.30-12.45 *Colasanti M., Casadei M.,Polticelli F.,Venturini G., Musci G..,Persichini T. (Università Roma Tre)*
S-glutationilazione di metallotioneina in seguito a stress nitrosativo/ossidativo

12.45-13.00 *Cannino G., Ferruggia E., Rinaldi A.M.(Università Palermo)*
Possible mechanisms regulating the expression of nuclear and mitochondrial genes

13.00-14.30 **Pausa pranzo**

14.30-14.45 *Cannino G. Ferruggia E., Luparello C. Rinaldi A.M. (Università Palermo)*
Effetti del cadmio sull'attività mitocondriale e sulla espressione genica in cellule MDA-MB231 di tumore mammario

14.45-15.00 *Menegola E., Broccia M.L., Di Renzo F., Giavini E. (Università Milano)*
Effetti di metionina ed acido valproico sullo stato di acetilazione e metilazione istonica embrionale: studio del possibile meccanismo di teratogenesi correlato a VPA

15.00-15.15 *Trinchella F., Cannetiello M., Simoniello P., Filosa S., Scudiero R. (Università di Napoli Federico II)*
Alterazione genica differenziale in embrioni di lucertola esposti al cadmio

15.15-15.30 *Di Renzo F., Broccia M.L., Menegola E., Giavini E.(Università Milano)*
Apoptosi come probabile via patogenetica associata alle malformazioni

allo scheletro assile indotte da acido valproico nel topo

15.30-15.45 *Benedetti E., Renato G. , D'Angelo B., Cinque B., Ricci A., Laurenti G., Lombardi D., Cifone M. G., Cimini A. (Università L'aquila)*
Caratterizzazione biomolecolare di cellule di glioblastoma umano in coltura primaria. Effetti differenzianti e anti-angiogenetici dei ligandi del PPARγ

15.45-16.00 *Malagoli D., Boraldi F., Annovi G., Quaglino D., Ottavini E. (Università di Modena e Reggio Emilia)*
Autofagia e morte cellulare programmata nella linea cellulare di insetto IPLB-LdFB: nuovi indizi da indagini di proteomica

16.00-16.15 *Sirchia R., Longo A., Luparello C. (Università Palermo)*
Regolazione dell'espressione di geni correlati all'apoptosi in cellule di tumore mammario umano coltivate su substrati di collagene tipo V

16.15-16.30 *D'Angelo B., Benedetti E., Di Loreto S., Cristiano L., Cerù M. P., Cimini A (Università L'Aquila)*
Vie di trasduzione del segnale coinvolte nel differenziamento neuronale indotto dal PPARß

16.30-16.45 *Candiani S., Moronti L., Pennati R, De Bernardi F., Benfenati F., Pestarino M. (Università Milano)*
Clonaggio e analisi del pattern d'espressione dei geni delle sinapsine in due protocordati: *Branchiostoma lanceolatum* e *Ciona intestinalis*

16.45-17.00 *Turturici G., Geraci F., Candela ME., Giudice G. and Sconzo G. (Università Palermo)*
Proteina Hsp70: ruolo intracellulare ed extracellulare

1700 -17.30 **pausa caffè**

Serata libera

Sabato 7 giugno
(Roma Tor Vergata - VILLA MONDRAGONE)

8.00 Servizio navetta da Globus Hotel a Villa Mondragone

Regolazione genica e differenziamento
Moderatori: A.M. Polzonetti-Magni e M. Miranda

9.30-9.45 *Polzonetti-Magni A.M., Palermo F., Cottone E., Virgili M., Mosconi G., Franzoni M.F. (Università Camerino)*
Endocannabinoidi e stress in pesci teleostei

9.45-10.00 *Piergentili R., Ficca A.G., Caccia E., Ceccarelli G., Missinato M.A., Mastrolia L.,Romano N. (Università Tuscia – Viterbo)*
Clonaggio ed espressione del gene catena gamma del recettore dei linfociti T nella spigola, *Dicentrarchus labrax*

10.00-10.15	*Caccia E., Vasta G.R., Ficca A.G., Strickler-Dinglasan P., Romano N. (Università Tuscia – Viterbo)* Developmental expression of antimicrobial peptides in zebrafish (*Danio rerio)*
10.15-10.30	*Zarivi O., Bonfigli A., Colafarina S., Cesare P., Miranda M. (Università L'Aquila)* Analisi trascrizionale dell'espressione del gene della tirosinasi nello sviluppo di *Bufo bufo*
10.30-10.45	*Picchietti S., Belardinelli C., Taddei A. R., Fausto A. M., Rossi M. Giorgi F. (Università Pisa)* *Internalizzazione* del recettore TSH e della Tireoglobulina in cellule tiroidee
10.45-11.00	*Accordi F., Chimenti C., Gallo V.P., Liguori R., Manelli H. (Università Roma La Sapienza)* Differentiation of interrenal cells in the developing adrenal gland of *Testudo hermanni*
11.00-11.30	**pausa caffè**
11.30-11.45	*Nardi A., Pomari E., Colombo L., Dalla Valle L. (Università Padova)* Controllo trascrizionale dell'enzima steroide solfatasi umana
11.45 -12.00	*Vicario A., Baj G., Leone E., Chao M., Tongiorgi E. (Università di Trieste)* Traffico cellulare del BDNF e citoarchitettonica dei neuroni
12.00-12.15	*Gargioli C., Cannata S.M, Bernardini S., Brunelli S., Cossu G.* (Parco Scientifico San Raffaele, Roma) sFRP-3 lega EGF nello spazio extracellulare: effetti sulla proliferazione, differenziamento e morfogenesi embrionale

Note 1) si raccomanda di rispettare i tempi previsti dal programma. 2) per le comunicazioni sono previsti 15 minuti, compresa la discussione. 3) I relatori non "geini" devono essere presentati da un Membro GEI

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Non solo Mauthner:
70 anni di ricerche di neurobiologia di Alberto Stefanelli

ERNESTO CAPANNA
ernesto.capanna@uniroma1.it
Dipartimento di Biologia Animale e dell'Uomo, Università di Roma La Sapienza,

Viene brevemente presentato un profilo delle ricerche neurologiche e neuro-embriologiche di Alberto Stefanelli che assommano ad oltre 150 contributi.
Il suo primo lavoro, del 1931, tratta del sistema nervoso periferico dei Ciclostomi in un clima culturale ove era ancora aperto il dibattito tra sistema nervoso reticolare e teoria del neurone. Con stringente logica consequenzialità, e grande rigore scientifico, da quel primo lavoro Stefanelli passa a studiare le strutture basali meso-diencefaliche dei Ciclostomi (1933-1939) ponendo particolare rilievo al sistema degli elementi reticolari, cellule e fibre di Müller. Rilevanti sono, nell'ambito di queste ricerche, le osservazioni sul numero e la grandezza dei neuroni giganti di Müller ove riconosce una costanza del loro numero in tre specie di differente taglia, *Petromyzon marinus*, *Lampetra. fluviatilis* e *Lampetra planeri*, e, come previsto dalla "legge di Levi", una diretta proporzionalità tra dimensione del pericarion e territorio innervato. Rimanendo nell'analisi delle strutture rombencefaliche, egli affronta il problema del "cervelletto" dei Ciclostomi che dà origine ad una serie di importanti lavori (1935-1943) sul cervelletto di Anamni e Amioti. Il metodo che Stefanelli utilizza in queste ricerche, metodo che egli chiama "*neuro-ecologico*" si fa carico di considerazioni comportamentali ed ecologiche oltre che strettamente neuro-anatomiche. Egli applica questo suo approccio in numerose ricerche, auali quelle sull'apparato stato-acustico dei Chirotteri in rapporto alle modalità di volo e di ricezione degli echi, sulla cito-architettonica del tetto mesencefalico nei pesci ciechi (*Astyanax*), e sui centri dei nervi oculomotori in rettili (*Lacerta* vs. *Chamaeleo*), ed altre ancora. Anche la presenza o assenza della cellula di Mauthner nelle forme adulte degli Osteitti viene letta in termini di interpretazione neuro-ecologica, confrontando specie pelagiche e demersali.
Prende così l'avvio di quella linea di ricerca che Stefanelli porterà avanti per l'intera seguente sua attività di ricerca; il neurone Mauthner nei pesci ossei e negli anfibi. Il neurone di Mauthner per Stefanelli è un neurone "larvale" tanto negli anfibi quanto nei pesci ossei. Da queste osservazioni e da esperienze di rigenerazione di strutture neurali scaturisce un importante pensiero teorico di Stefanelli, quello dei ciclo vitale dei neuroni (1951-1960).
La coppia di cellule di Mauthner degli Anfibi offre a Stefanelli, a partire dal 1944, la possibilità di affrontare con metodo sperimentale, con i limitati metodi ai tempi disponibili, non più che trapianti ed espianti, il problema della induzione, determinazione e differenziamento della coppia di neuroni di Mauthner. Riesce così a stabilire i tempi precoci della sua determinazione irrevocabile già allo stadio di placca neurale. In quelle stesse esperienze riesce a porre in evidenza quali siano i fattori che influenzano l'orientamento e la decussazione delle fibre di Mauthner. I problemi dell'orientamento delle fibre nervose nella neuroistogenesi, così come dello stesso differenziamento neuronale, in condizione di espianto e/o di coltura un vitro,di neuroblasti nei neuroni specificamente determinati occuperanno la ricerca di Stefanelli a partire dal 1954. Un intelligente approccio sperimentale è ideato da Stefanelli per affrontare il problema dello "*autonomo differenziamento*" di neuroblasti che avessero già avuto una loro determinazione, ma non fossero ancora differenziati Dapprima, in espianti in allanto-corion (1954) osserva, in completa assenza dell'apporto di fibre dalla periferia e da centri correlati, un regolare differenziamento dei neuroni fusiformi a neurite ricorrente del tetto ottico degli uccelli, e dei neuroni di Purkinje del cervelletto. Mette poi a punto una più affinata tecnica basta sulla coltivazione *in vitro* di sferule originate da spontanea riaggregazione di cellule embrionali di pollo

disgregate provenienti dall'abbozzo dell'occhio e da altri distretti archiencefalici. Questi riaggregati, di cellule tratte da vescicole ottiche di embrioni di 10 giorni, analizzati al microscopio elettronico, mostrarono la formazione delle specifiche sinapsi, quali quelle tra fotorecettori e cellule del ganglio retinico. Ancor più interessante è quanto si osserva in sferule originate dalla riaggregazione di abbozzi del cervelletto (1977). In questo caso si osserva che peculiari sinapsi, con le stesse caratteristiche osservabili *in vivo* si formano in completa assenza delle afferenze nervose che le determinano *in vivo*, dimostrando così che la formazione di tipici apparati sinaptici sono espressione della autonoma capacità differenziativa dei neuroni post-sipaptici.
Nei suoi lavori più recenti Stefanelli ritorna a precisare con la microscopia elettronica sue antiche osservazioni, come quelle sul nucleo vestibolare della lampreda (1937) che descrive ora dettagliatamente in un lavoro 1968. Analogamente torna con osservazioni al microscopio elettronico ad indagare sul problema del significato funzionale della cellula di Mauthner negli Osteiti analizzando i dettagli l'apparato sinaptico dell'*axon-cap*.

Reticulospinal Neurons in Anamniotic Vertebrates: A Celebration of Alberto Stefanelli's Contributions to Comparative Neuroscience

STEVEN J. ZOTTOLI, CARLA CIONI AND ERNST-AUGUST SEYFARTH
carla.cioni@uniroma1.it

Over the past 76 years Alberto Stefanelli has successfully used a comparative approach to study the nervous system. His main research focus during that time has been on identifiable reticulospinal neurons including Müller and Mauthner neurons found in anamniotic vertebrates.
His seminal work on the constancy in number and position of giant identifiable reticulospinal neurons in the brains of larval and adult lampreys, and his assertion that only a subset of these neurons were Müller cells, provided the framework in which subsequent authors have refined our understanding of the cellular anatomy, axonal projections, physiology, and function of Müller cells in the control of movement. Stefanelli has also provided the most comprehensive study to date of the Mauthner cell and its axon cap. His description of the differences in axon cap structure among many fishes and amphibians and his use of the "morpho-ecological" approach to determine Mauthner cell function has provided the basis for future studies on the neuronal basis of behavior and its evolution.
In the occasion of Professor Stefanelli 100th birthday, we celebrate his scientific contributions to comparative neuroscience with an overview of his scientific accomplishments and our view of how his comparative studies continue to contribute to our understanding of the nervous system.

Neural precursors and their ability to respond to induction of dopaminergic differentiation

GABRIELLA AUGUSTI-TOCCO
gabriella.tocco@uniroma1.it
Dipartimento di Biologia Cellulare e dello Sviluppo. Università di Roma La Sapienza

Data on the persistence of stem cells in the adult nervous system have been reported over the last decade (Temple, 2001); these findings have aroused great interest also for the possible development of therapeutic strategies in the treatment of neurodegenerative diseases . On the other hand a number of questions have been posed, which remain by and large open, regarding the plasticity of adult stem cells and/or neural precursors residing in different regions of the nervous system. This is a particularly relevant questions, since in each neurodegenerative disease a specific neuron type is lost and need to be regenerated.
Several genes have been discovered, which take part in the morphogenesis of the nervous system and neuron differentiation (Bertrand et al., 2002; Kiecker and Lumsden, 2005; Singh

et al., 2008); on the other hand genes responsible for the specification of different specific neuronal populations are less characterized; their characterization would be of major relevance in order to direct stem cell differentiation along differentiaton pathways of interest.

Nurr1 belongs to the orphan nuclear receptor family and has been reported to play a role in the specification of midbrain dopaminergic neurons (Volpicelli et al., 2004). Its expression in ES cells (embryo derived stem cells) has been reported to promote dopaminergic neuron differentiation (Chung et al., 2002).

Nurr1 thus appears as a good candidate to test the ability of neural precursors derived from various brain region to be directed to a dopaminergic fate, as compared to ES uncommitted stem cells.

We have then used

- the ES-D3 mouse stem cell line, used in previous studies
- stem cell lines derived from cortex, striatum, mesencephalon and spinal cord of E 13.5 mouse embryos, as well as neural precursors derived from adult SVZ.

Nestin positive neural precursors derived from the NS or obtained from ES-D3 cells, following a described protocol, have been subjected to lentiviral infection to induce Nurr1 expression.

The expression of neuronal and dopaminergic markers has been evaluated by RT-PCR and immunostaining.

In Embryonic stems cells (ES-D3), as previously shown, the forced expression of Nurr1 induces tyrosine hydroxylase (TH) expression. The same results were observed in neural precursors obtained from E13.5 striatum and mesencephalic precursors, with a significant increase of TH positive neurons in infected cultures. On the other hand in neural precursors isolated from cortex and spinal cord no TH positive neurons were found after Nurr1 overexpression. Adult SVZ precursors also do not respond to Nurr1 with activation of TH expression. These data suggest that Nurr1 ability to induce a dopaminergic phenotype is related to the brain region of cell derivation and that precursors ability to respond to Nurr1 is regulated during development possibly through regionalizing processes.

Bertand N., Castro D.S., Guillemot F. (2002) Nature Rev Neurosci, 3: 517-30

Chung S, Sonntag KC, Andersson T, Bjorklund LM, Park JJ, Kim DW, Kang UJ, Isacson O, Kim KS (2002) Eur J Neurosci ,16:1829-38

Kiecker C. and Lumsden A (2005). Nature Rev Neurosci, 6: 553-64

Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S (2008) Nature, 453:223-29

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The long migratory journey of GnRH-1 Neurons

PAOLO GIACOBINI AND ALDO FASOLO,
Dept. Animal and Human Biology, University of Torino
aldo.fasolo@unito.it

GnRH-1 (Gonadotropin Releasing Hormone-1) cells arise in embryonic life from an extra-CNS region (namely the olfactory placode) and migrate along olfactory/vomeronasal axons into the brain to become integral members of the hypothalamic-pituitary-gonadal axis. Once GnRH-1 neurons have reached the hypothalamus, they project the axons to the median eminence, where they release the hormone into the pituitary portal vessels to induce the secretion of the anterior pituitary gonadotropins into the general circulation. Disruption of either the development or regulation of the GnRH-1 system results in reproductive dysfunctions.
Although the migration of GnRH-1 cells from nose to brain has been documented across vertebrates, many questions remain concerning the molecules and cues directing this migratory process and olfactory axon guidance. The development of the olfactory system and the GnRH-1 system are intimately entwined and if the course of olfactory axons is altered, the migration of GnRH-1 cells is impaired. The developmental relationship of these systems is well illustrated by human Kallmann's syndrome (KS). KS is an inherited disorder characterized by the association of hypogonadotropic hypogonadism (HH) and anosmia. Examination of fetal material revealed lack of olfactory axon ingrowth into the forebrain and GnRH-1 cells "stuck" on the dorsal side of the cribriform plate. Therefore, the current hypothesis is that KS is the result of a defect in migration of both olfactory nerves and GnRH-1 neurons. The cues that direct these axons and these cells have not been completely identified but may be critical for guidance to the appropriate tissue targets. A variety of molecules are expressed on olfactory/vomeronasal axons, but to date none has exclusively been limited to the GnRH-1 cell route or been shown to have a direct role in GnRH-1 cell movement/guidance. Certainly our understanding of GnRH-1 cells establishes conditions for understanding mechanisms controlling reproductive development, both activation and failure. In addition, the ability to manipulate the molecular and cellular biology of this system opens the route examining neurobiological issues such as phenotypic commitment, axon outgrowth and neuronal migration. The unraveling of these pathways is providing novel insights into genes that, if disrupted, might result in a phenotype of HH or KS.
During last 15 years, there have been a variety of studies looking at factors that modulate GnRH-1 neuron migration. Most of these studies have described factors that modify the rate of migration. Most recently, soluble factors have been included to the list of molecules influencing the development of the GnRH-1 system. Among these, hepatocyte growth factor, HGF, and secreted-class 3 semaphorins, Sema 3A and 3F, have been shown to play important roles in the control of GnRH-1 migratory process. HGF is a cytokine which, acting via its receptor Met, exhibits mitogenic, motogenic and chemoattractive activities in neuronal and non-neuronal cells. HGF and Met are widely distributed in developing brain, however, few studies address the function(s) of Met signaling during brain development. HGF is expressed in nasal embryonic mesenchyme with an increasing gradient toward the border between the nose and telencephalon with an expression pattern correlating with GnRH-1 neuronal migration. We have shown that HGF exerts motogenic and chemotactic effects on both immortalized and primary GnRH-1 cells, acting to positively modulate cell-cell and cell-ECM interactions during the early aspects of their migratory process. Semaphorin, plexins and Met receptor are independently identified protein families that share structural similarities. Semaphorins are a wide family of secreted and membrane-bound proteins that play a critical role in cell migration and axon guidance in selected areas of the developing and adult nervous system. In particular, certain membrane-bound semaphorins, like

Sema 4D, are proteolytically released in the extracellular space and such molecules diffuse in the surrounding area and act upon cells expressing the specific receptors (Plexins). The biological activity of Sema 4D is mediated by three receptors, Plexin B1, the high-affinity receptor widely expressed throughout different tissues, including CNS, CD72, a low-affinity receptor found in the hemangiopoietic lineage, and Plexin B2 which is expressed in the peripheral and central nervous system. Plexin B1 can also interact with the receptor tyrosine kinases Met and Sema 4D, depending on the cellular context and on the differential association of Plexin B1 with Met, can exert opposing effects. We have recently shown that GnRH-1 cells express the receptor Plexin B1, but not Plexin B2, in a temporal window associated with their migratory process, whereas Sema 4D expression is distributed along the GnRH-1 migratory route with a peak of expression in the hypothalamic target area. Analysis of Plexin B1 deficient mice revealed a migratory defect of these cells leading to a reduced size of GnRH-1 neuronal population in adult brains. Using different experimental approaches, we demonstrated that Sema 4D promotes the migratory activity of immortalized GnRH-1 cells through the activation of Plexin B1 and of the associated Met tyrosine kinase receptor.
Taken together, our data reveal a novel function of Sema4D in the regulation of GnRH-1 neurons development, and identify Plexin B1-Met receptor complex as a fundamental asset for neuronal cell migration and guidance.
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Origine, potenzialità e destino differenziativo dei mesoangioblasti, progenitori meodermici della parete vascolare.

GIULIO COSSU
cossu.giulio@hsr.it
Istituto di Ricerca per le Cellule Staminali, San Raffaele e Dipartimento di Biologia, Università di Milano.

Lo sviluppo fetale e post-natale del mesoderma avviene, con l'eccezione del sangue, per accrescimento di tessuti quali osso, muscolo scheletrico e cardiaco, costituiti da cellule che proliferano poco o nulla. Pertanto, nuove cellule devono essere incorporate nei tessuti a partire da progenitori ancora mitotici quali gli osteoblasti del periostio o le cellule satelliti del muscolo scheletrico che devono regolare la loro attività mitotica così da produrre cellule pronte a differenziare e alter cellule progenitrici, almeno fino alla fine dell'accrescimento somatico post-natale. Poiché questi tessuti sono anche capaci di riparare danni conseguenti a traumi o patologie genetiche o acquisite, ne consegue che almeno una parte di progenitori deve persistere per tutta la vita (Cossu & Bianco, 2003) .
Anni fa, isolammo e caratterizzammo progenitori associati alla parete dei vasi sanguigni embrionali e capaci di proliferare in vitro e differenziare nei principali tipi di mesoderma solido (Minasi et al. 2002). Per tali motivi definimmo queste cellule "mesoangioblasti" e ipotizzammo che progenitori vascolari potessero essere reclutati al destino differenziativo dei tessuti in cui il vaso sanguigno penetra durante l'angiogenesi fetale. Successivamente, mesoangioblasti sono stati isolati da vasi sanguigni embrionali e post-natali di tessuti murini, umani (Dellavalle et al. 2007) e di altre specie. Le cellule isolate dall'embrione presentano caratteristiche di endotelio immaturo mentre le cellule dai tessuti adulti presentano caratteristiche di periciti. Benché mesoangioblasti siano stati utilizzati con successo in modelli pre-clinici di terapia cellulare per le distrofie muscolare (Sampaolesi et al. 2003, 2006), non molto è

noto sulla loro origine embrionale, sul loro rapporto genealogico con i progenitori vascolari (Esner et al. 2006) e del loro destino naturale durante lo sviluppo pre- e post-natale del mesoderma.
Per rispondere a queste domande abbiamo condotto una serie di esperimenti mediante colture di organo, analisi clonale retrospettiva e marcatura genetica dei mesoangioblasti. I risultati ottenuti finora indicano che periciti dei piccoli vasi del tessuto muscolare striato (scheletrico e cardiaco) contribuiscono alla formazione di fibre muscolari scheletriche e cardiomiociti (rispettivamente) durante lo sviluppo post-natale. La possibile origine embrionale e possibili rapporti genealogici con le cellule residenti nel muscolo embrionale saranno discussi.

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The oncogenic factor TCL1 regulates the blastomere proliferation of early mouse preimplantation embryos by interacting with the AKT2 isoform of protein-kinase PKB/AKT

FRANCO MANGIA
franco.mangia@uniroma1.it
Department of Psychology, Section of Neuroscience, Istituto Pasteur – Fondazione Cenci Bolognetti and Research Center "Daniel Bovet", La Sapienza University of Rome

In all animals, fertilization triggers a complex developmental program based on the shift of egg transcriptional activity from a maternal to a zygotic pattern. In mammals, this transition is mediated by an extensive zygotic genome assembly into chromatin and the appearance of novel, gene-specific mechanisms of trancriptional regulation. Besides the reprogramming of gene expression, fertilization also allows the egg to overcome the metaphase II block of meiosis and then to undergo a very rapid series of mitotic divisions. In vertebrates, including mammals, embryo early blastomere proliferation displays the peculiar feature of a very short G1 phase of the cell cycle. However, molecular mechanisms that regulate this kind of cell proliferation are still largely unkown. We have been studying the molecular and cellular regulation of preimplantation development since many years in the mouse. More recently, we have focused our attention on the role that, besides lymphoid cells, the oncogenic T-cell Leukemia Factor 1 (TCL1) plays in mammalian oocytes and preimplantation embryos (Narducci et al., 2002). The interest in TCL1 has recently been strengthened by the finding that this factor is also expressed in embryonal stem cells of mammals, being activated downstream from *Oct4* and representing a major player in the balance between proliferation and differentiation of these cells (Matoba et al., 2006). It is commonly accepted that TCL1 promotes cell proliferation by physically interacting with Protein Kinase B (PKB/AKT). Heterodimerization with TCL1 triggers the AKT recruitment to the plasma membrane, thus allowing AKT transphosphorylation at the serine 473 residue and the subsequent transfer of phosphorylated AKT to the nucleus. These conclusions, however, were mostly obtained using *in vitro* cultured cell lines overexpressing TCL1/AKT and thus they not necessarily are representative of actual *in vivo* functions of these factors.

We have studied the regulatory role that TCL1 plays on early blastomere proliferation during mouse preimplantation development, with particular reference to TCL1 expression and subcellular localization, as well as the interaction this factor establishes with AKT isoforms AKT1, AKT2 e AKT3, having idiosyncratic but still largely unkown functions. Using a *Tcl1*-KO mouse

strain and the intracytoplasmic microinjection of inactivating antibodies to TCL1, AKT isoforms and several factors/kinases of the signalling cascade downstream from PI3K/AKT, we show that TCL1 is required for the nuclear translocation of AKT2, but not AKT1 nor AKT3, and that the AKT2 nuclear transfer is in turn necessary for the normal mitotic progression of preimplantation embryos (Fiorenza et al., 2007).

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Expression of *Djmot*, a member of the HSP70 multi-gene family, is required for stem cell proliferation in planarians

MARIA CONTE, PAOLO DERI, LINDA MANNINI, RENATA BATISTONI
mconte@biologia.unipi.it
Dipartimento di Biologia, Università di Pisa

In adult organisms stem cells are essential to homeostasis and regeneration of damaged tissues. This means that stem cells should be equipped with efficient cytoprotective mechanisms to respond to environmental and physiological stresses. Planarians (Platyhelminthes) are a model system well known for regenerative potential, high body plasticity and continuous turnover of all differentiated cell types. These characteristics are due to the presence of a population of pluripotent stem cells, called neoblasts. Damage or reduction in the number of these cells deeply affects planarian regeneration and survival. Heat shock proteins (HSP) have cytoprotective effects and their expression may be induced in response to a wide variety of stress conditions. In a preliminary analysis we noted that some genes coding for different HSP members are specifically expressed in neoblasts, suggesting that these proteins play important roles in cytoprotection of these cells. In particular we focused our study on a specific gene (*Djmot*) encoding a protein belonging to HSP70 family, which has a strong similarity with mammalian mortalin (also known as Grp75, PBP74, mthsp70). As demonstrated by in situ hybridization experiments, transcripts of *Djmot* appeared widely distributed in the parenchyma of the planarian body, with a pattern that reflects the distribution of neoblasts. A drastic reduction of the parenchymal *Djmot* expression was also observed in planarians irradiated with a dose of X-rays (30Gy) that causes selective death of these cells. Functional studies by RNA interference (RNAi) indicate that *Djmot* expression is essential for the proliferative activity/survival of neoblasts. In fact, after injection with *Djmot* dsRNA, planarians became unable to regenerate or formed a small and abnormal blastema. A substantial diminution of mitotic divisions of planarian stem cells was also observed. Finally, most of the *Djmot* RNAi-injected animals died within 1-2 months.

How to drive *Xenopus* embryonic stem cells toward a retinal fate

SIMONA CASAROSA, ANTONIO VITOBELLO, LAN LEI, MASSIMILIANO ANDREAZZOLI, FEDERICO CREMISI, ROBERT VIGNALI, GIUSEPPINA BARSACCHI
scasarosa@biologia.unipi.it
Biology Department, Cell and Developmental Biology Unit, University of Pisa, Pisa, Italy

Cell-replacement therapies are among the approaches to cure retinal degenerative diseases, such as Retinitis pigmentosa. In these therapies, the degenerating cells are replaced with stem cells that have been appropriately differentiated *in vitro*. Currently, the crucial phase is the differentiation step, as none of the reported protocols leads exclusively to a retinal fate.
In the past years we have gained an extensive knowledge regarding a class of transcription

factors conserved among vertebrates that play a crucial role in retinal development. We and others have shown that a cocktail of seven homeobox transcription factors is able to convert non-neural ectoderm into a complete eye. Therefore, these genes (Eye Field Transcription Factors, EFTFs) appear to be top choice candidates to direct stem cells differentiation toward retinal fates. In many respects *Xenopus* animal cap cells can be considered equivalent to mammalian embryonic stem cells. In animal cap assays, the overexpression of the neural inducer noggin activates six of the seven EFTFs. We have tested the capability of noggin to drive *Xenopus* animal cap embryonic stem cells toward retinal fates. By RT-PCR analysis, we show that noggin is able to activate the expression of many retinal genes. Moreover, it has a dose-dependent effect in the activation of genes involved in the terminal differentiation of specific retinal cell types.
Finally, we tested the ability of noggin-expressing animal caps to rescue eye development in vivo. After removing half of the eye field from neurula stage *Xenopus* embryos, we replaced it with an animal cap expressing a low or high dose of noggin. The high noggin dose animal caps are able to rescue the formation of an eye in 95% of the cases. These eyes show a correct morphology, lamination and histology of the retina and correctly express cell type-specific markers. On the other hand, the low noggin dose animal caps rescue eye development in only 27% of the cases. These eyes have a poorer morphology and are less well developed.
We have thus shown that high doses of noggin are able to drive the expression of retinal differentiation markers in *Xenopus* animal cap embryonic stem cells, and that the noggin expressing stem cells are able to rescue eye development, at least in a proper embryonic environment.

Gamma amino butyric acid (GABA) receptors in induced neuron-like Bone Marrow Stromal Cells

SIMONA CANDIANI[1], MANUELA MARCOLI[2], LAURA TONACHINI[3,4], MASSIMILIANO MONTICONE[3,4,5], MADDALENA MASTROGIACOMO[3,4], ANDREA OTTONELLO[3,4], CHIARA CERVETTO[2], PAOLA PALUZZI[2], GUIDO MAURA[2], RANIERI CANCEDDA[3,4], PATRIZIO CASTAGNOLA[4], MARIO PESTARINO[1]
pesta@unige.it
[1]Dept. of Biology, University of Genova, Italy
[2]Dept. of Experimental Medicine, Section of Pharmacology, University of Genova, Italy
[3]Dept. of Oncology, Biologye and Genetics, University of Genova, Italy
[4]Istituto Nazionale per la Ricerca sul Cancro (IST), Genova
[5]Advanced Biotecnology Center (ABC), Genova

Bone marrow stromal cells (BMSC) are pluripotent cells that, after exposure to an inductive medium, show a neuronal-like morphology. These induced BMSC derived neuron-like (BDNL) cells express several neuronal markers such as Microtubule Associated Protein Tau, Neurofilament M, and Nestin as revealed by semiquantitative PCR and immunocytochemistry. In order to verify if the BDNL are functionally modified, we have focused our attention on the expression of neurotransmitter receptors. In particular, we have found a greatly enhancement of the expression of $GABA_A$ subunits α1, ..β2/3, ε and $GABA_{B1}$ and $GABA_{B2}$ mRNAs in BDNL. Similar results were obtained from rat skin fibroblasts subjected to the same induction protocol, with the exception for the $GABA_{B2}$ transcript that was expressed only by BDNL. The presence of both $GABA_{B1}$ and $GABA_{B2}$ subunits in BDNL cells suggests that functional $GABA_B$ receptors might be assembled. By pharmacological approach we demonstrated that a functional $GABA_B$ receptor, negatively linked to cyclic AMP production, was present in BDNL. Therefore, we suggest that BMSC can be converted into cells provided with appropriate receptors coupled to transduction

mechanisms and potentially responding to a specific neurotransmitter. (Grant from MIUR-PRIN 2006058952)

Development and regression of nervous system in *Botryllus schlosseri* (Ascidiacea).

LUCIA MANNI, GIOVANNA ZANIOLO AND PAOLO BURIGHEL
lucia.manni@unipd.it

Among chordates, colonial tunicates offer the unique possibility to study organ ontogenesis in various developmental pathways, i.e. sexual and asexual reproduction. Actually, the fertilized egg develops into a swimming larva with a chordate body plan. The larva, then, undergoes metamorphosis and differentiates into a sessile oozooid, the founder of a new colony, which grows producing, asexually, numerous blastozooids. In *Botryllus schlosseri*, the colony growth occurs by cyclical series of generations, with synchronized development, functional activity and dead of blastozooids of a same generation. In this ascidian, we have studied by cytochemistry and electron microscopy, the development of nervous system, both during embryogenesis and blastogenesis. In the embryo, it forms from a typical neural plate, which rolls up in a neural tube and, in the larva, differentiates in an anterior cerebral vesicle, containing a photolith for perception of both gravity and light, a cerebral ganglion and a nerve cord. At metamorphosis, this nervous system regresses completely by apoptosis, while the neural complex of adult develops from a remnant of the original neural tube, the neurohypophysial duct. The neural complex is constituted of a neural gland, direct derivation of the duct, and an ovoid cerebral ganglion, originated by delamination of neuroblasts from its wall. A similar neural complex is formed, with different modalities, also during blastogenesis. Here, it derives from a tubular structure, called dorsal tube, which forms from a vesicle of multipotent somatic cells. Gradually, the tube becomes the neural gland while its wall proliferates neuroblasts, which coalesce to organize the cerebral ganglion. A complex nerve network extends progressively in the bud and a strict temporal relation links the development of the local neural network with its target differentiating organ. The pattern of innervation undergoes dynamic rearrangements; a number of nerves emerge from the ganglion, but a marked process of axon elimination takes place when the fully-developed bud enters its functional activity. The final pattern of innervation seems to be regulated by axon withdrawal, rather than apoptosis of neurons. At the end of the blastogenic cycle, the neural complex is resorbed by apoptosis. We discuss similarities and differences found in the ontogenetic processes underlying the construction and regression of the nervous system during embryogenesis and blastogenesis.

Characterisation of progranulin in the central nervous system of the leech *Hirudo medicinalis*

JACOPO VIZIOLI, FRANÇOISE CROQ, CHRISTOPHE LEFEBVRE, MICHEL SALZET, JOËL PESTEL & PIERRE-ERIC SAUTIÈRE,
jacopo.vizioli@univ-lille1.fr
Laboratoire de Neuroimmunologie des Annélides - FRE 2933 CNRS. Université de Lille 1- 59655 Villeneuve d'Ascq, France

Progranulin (PGRN) is a pluripotent growth factor expressed in many animal groups and involved in development, inflammation, wound repair and tumorigenesis. In Mammals, the progranuline gene encodes a cystein rich glycoprotein containing 7.5 repeated motifs called granulins (GRN). Granulins are 6 kDa peptides produced by proteolytic cleavage of the precursor PGRN. In central nervous system (CNS) this molecule is expressed by neurons and microglial cells but its function is little known. PGRN recently gained the attention of the neuroscience community with the discovery that mutations in the gene are linked to frontotemporal lobar degeneration and other neurodegenerative pathologies.
To better understand the function of (pro)granulins in CNS, we investigate the role of this molecule in an invertebrate model, the

medicinal leech *Hirudo medicinalis*. This animal has a relatively simple CNS organisation and its nerve cell physiology is well known. In addition, the medicinal leech is able to repair its injured CNS, getting a complete functional recovery in a few weeks after lesion. These elements make this annelid a recognized model in neurobiology.
The complete coding sequence of *Hirudo* progranulin (Hm*PGRN*) gene has been cloned. It codes a 150 kDa protein and shows a high similarity with human and other animal progranulins, *Hm*PGRN contains 15.5 putative granulin motifs, and it constitute the richest GRN containing precursor so far described in animal kingdom. Hm*PGRN* gene is constitutively expressed in nerve cord and it is upregulated during CNS regeneration. Immunohistochemistry analysis revealed the presence of the (pro)granulin in neurons cell bodies, showing an accumulation of the protein after injury. These data suggest that *Hm*PGRN may have a neurotrophic role and contribute to the repair process.
Since progranulin structure is highly conserved in animals, the study of this molecule in leech might increase our knowledge on its functions in normal or injured CNS and have major therapeutic interests.

Effetti opposti dell'Acetilcolina sulla proliferazione e il differenziamento di cellule gliali mielinizzanti.

ADA MARA TATA, SIMONA LORETI, FEDERICA DE ANGELIS, *SIMONA LO RETI, ANTONELLA BERNARDO, §VALERIO MAGNAGHI, *LUISA MINGHETTI E GABRIELLA AUGUSTI TOCCO
adamaria.tata@uniroma1.it
Dip. di Biologia Cellulare e dello Sviluppo, Università di Roma "La Sapienza"; *Dip. di Neurobiologia, ISS,Roma, § Dip. di Endocrinologia, Università di Milano

Nel sistema nervoso le cellule gliali e neuronali comunicano sin dalle prime fasi dello sviluppo mediante fattori di crescita, molecole di adesione e neurotrasmettitori. Questi ultimi, in siti extra-sinaptici, assumono il ruolo di molecole di segnale intercellulare, modulando così la proliferazione e il differenziamento neuronale e gliale. Le cellule di Schwann e gli oligodendrociti, popolazioni glali responsabili della melinizzazione degli assoni, esprimono recettori per diversi neurotrasmettitori tra cui l'acetilcolina. Da studi in vitro è emerso che la selettiva attivazione del sottotipo muscarinico M2 comporta un arresto reversibile della proliferazione delle cellule di Schwann. L'uscita dalla fase proliferativa determina una spinta nel loro programma differenziativo come indicato dai vistosi cambiamenti morfologici osservati dopo trattamento con l'agonista M2, insieme con la variata espressione e distribuzione di proteine del citoscheletro e della superficie cellulare (es. N-CAM e N-Caderine) e dall' incremento di espressione di proteine della mielina (P0, PMP22).
Studi condotti su colture primarie di progenitori oligodendrocitari (OPC) hanno indicato che l'ACh ha su queste cellule effetti opposti a quelli osservati sulle cellule di Schwann. In questo caso il trattamento delle cellule con agonisti colinergici causa un aumento della proliferazione cellulare, che risulta essere mediato prevalentemente dai sottotipi muscarinici M3 e M1. Il trattamento con agonisti colinergici induce l'incremento della espressione del recettore per il PDGF , fattore di crescita richiesto per stimolare la proliferazione dei OPC, suggerendo che l'incremento della proliferazione indotto dall'ACh potrebbe essere favorito da una aumentata capacità di risposta delle cellule al loro fattore di crescita.
Il trattamento con agonisti colinergici è inoltre in grado di abbassare l'espressione per le proteine della mielina (es. MBP, CX32, MAG), sia nei OPC che negli OL maturi. Questi dati suggeriscono che l'ACh induce un incremento della proliferazione nei OPC e un ostacolo alla progressione nel loro programma differenziativo.
In conclusione i dati ottenuti nei due modelli cellulari dimostrano un effetto opposto dell'ACh nel controllo della proliferazione e del differenziamento delle cellule di Schwann e degli OL, effetti nei due casi mediati da differenti sottotipi recettoriali muscarinici.

Molecular mechanism of programmed cell death in aberrant multinucleated cells

ROBERTA NARDACCI [1], JEAN-LUC PERFETTINI[2], ALESSANDRA AMENDOLA[1], GUIDO KROEMER[2] E MAURO PIACENTINI[1,3].
nardacci@inmi.it

[1.] Istituto Nazionale per le Malattie Infettive « Lazzaro Spallanzani », Roma.
[2]. CNRS-UMR8125, Institut Gustave Roussy, Villejuif, France.
[3]. Dipartimento di Biologia, Università di Roma "Tor Vergata", Roma.

The generation of syncytia is a physiological process and aberrant syncytia are generated in some pathological conditions. Utilising a cellular model of HeLa cells stably transfected**,** we previously demonstrated that syncytia undergo apoptosis through a complex pathway involving several kinases (cyclin-dependent kinase-1, Cdk1; mammalian target of rapamycin, mTOR; p38 mitogen-activate kinase) and transcription factors (NF-kB; p53), resulting in the activation of p53 target genes Puma and Bax and triggering of the mitochondrial apoptotic pathway. All these factors have also been investigated *in vivo,* by analysing syncytia generated in several human tissues by HIV infection. To further characterise the HIV-induced apoptotic pathway we analysed the involvement of p53 binding protein-1 (53BP1) and ataxia teleangiectasia mutated (ATM) in HIV-induced apoptosis. 53BP1 is a nuclear protein involved in cellular response to DNA damage, and is phosphorylated by the PIKK family member ATM. We analysed the Peripheral Blood Mononuclear Cells (PBMCs) from uninfected, HIV-infected untreated and Highly Active Antiretroviral Therapy (HAART)-treated patients by immunocytochemical analysis. HIV-infected and untreated patients show high expression of 53BP1 and ATM and a significant reduction of both after HAART treatment. ATM and 53BP1 positively correlate with viremia demonstrating a modulation of these factors during the viral infection. Moreover, there is a significant correlation between the percentage of cells positively stained for 53BP1 and ATM. We also analysed paraffin sections from frontal cortex samples of 17 patients with HIV-associated dementia, thus revealing the expression of P53BP1 and ATM not only in HIV-induced brain syncytia but also in several neurons and glial cells. The analysis of lymph nodes from untreated HIV carriers revealed a high presence of the proteins in syncytia and in a fraction of mononuclear cells. These data indicate that the activation of 53BP1 and ATM occurs in cell death related to HIV infection providing substantial new information on the molecular mechanisms regulating the demise of aberrant syncytia.

Pre-adipocytes commitment to cholinergic neurogenesis

MARIA GRAZIA ALUIGI, ROSELLA CORADEGHINI*, CHIARA GUIDA*, CHIARA SCANAROTTI*, ANNA MARIA BASSI**, CARLA FALUGI, GRAZIA TAGLIAFERRO, SARA FERRANDO, PIERLUIGI SANTI*, EDOARDO RAPOSIO*.
falugi@unige.it
Università di Genova, Dip. Biologia (DIBIO); *-Dipartimento di Discipline Chirurgiche e Morfologiche e Metodologie Integrate (DICMI), ** Dipartimento di Medicina Sperimentale (DIMES), Italia.

The culture of mesenchyme derived precursors cells is of value both in the study of early human differentiation and in the creation of a source of donor cells for (self)-transplantation therapy. Here we report the presence and maturation of molecules related to the cholinergic and GABAergic neurotransmitter systems and related glial cells from primary cultures of human pre-adipocytes to precursors of neural cells. Along this pathway, acetylcholinesterase and choline acetyltransferase, the enzymes modulating the neurotransmitter acetylcholine, are expressed since the native cell stage, with different localizations and different roles along neural commitment. At the native cell stage and in controls, the enzymes are present, but do not retain catalytic activity, while the catalytic activity

appears only during the assumption of nervous-like structure, suggesting a parallel onset of function.

- Pre-adipocytes have been identified as a putative stem cell population within the adipose stromal compartment.
- The adipose tissue is a renewable tissue, containing stem cells, precursors of both fibroblast and fat cells. Like bone marrow, it originates from the embryonic mesenchyme and contains a stroma that is easily isolated.
- Pre-adipocytes, like bone marrow stromal cells (MSCs), are able to differentiate into multiple cell types of mesenchymal origin.

5-HT2B signalling participates in retinal and craniofacial morphogenesis during *Xenopus laevis* development

MICHELA ORI, ELISA REISOLI, STEFANIA DE LUCCHINI, IRMA NARDI
mori@biologia.unipi.it
Unità di Biologia Cellulare e dello Sviluppo, Dipartimento di Biologia, Università di Pisa, Via Carducci 13 Ghezzano, 56010 Pisa, Italia.

Serotonin (5HT) is a neurotransmitter that mediates a wide variety of effects in the central and peripheral adult nervous system. Experimental evidences demonstrated that serotonin even has an important role as growth and differentiating factor for neuronal and non-neuronal cells by controlling proliferation, migration and apoptosis during development. All the biological actions of 5-HT are mediated by G-coupled receptors and, among these, the 5-HT2B receptor is expressed during CNS, heart and craniofacial development. By using *Xenopus laevis* as a model system, we demonstrated that 5HT2B receptor loss of function determines a decrease in the proliferation rate of retinoblasts and increases the apoptosis of retinal cells thus resulting in abnormal eye morphology (De Lucchini et al., 2005). In order to further investigate 5HT2B role during development, we performed complementary experiments of gene gain of function. The overexpression of 5HT2B, leads to the formation of eyes with irregular form, position and orientation and showing defects in the optic fissure closure and in the pigmented epithelium formation. A detailed molecular analysis pointed out a disorganization of the typical laminar retinal structure and the presence of differentiated neuronal cells in ectopic position. Moreover, as pharmacological treatments with 5HT2 antagonists elicited in mice craniofacial alterations, we are studying the formation of craniofacial muscles and skeletal elements in both overexpressing and depleted 5HT2B embryos. In particular, alteration of 5HT2B expression during embryogenesis results in altered formation of extraocular muscles as well as of the jaw and hyoid cartilages and correlated muscles.

Conclusion: We showed that 5HT, *via* 5HT2B receptor, is among the key extracellular signals that control *Xenopus* retinal histogenesis and eye morphogenesis. Moreover our results indicate for the first time a direct involvement 5-HT2B receptors in mediating the serotonin action on craniofacial morphogenesis by influencing the formation of skeletal elements and that of the connected muscles.

De Lucchini S., Ori M., Cremisi F., Nardini M., Nardi I. (2005). 5-HT2B-mediated serotonin signaling is required for eye morphogenesis in Xenopus. *Mol Cell Neurosci.* 29(2): 299-312

Genes regulated by the transcription factor *Xrx1*: a microarray analysis

GUIDO GIUDETTI, MARTINA GIANNACCINI, DANIELE BIASCI, CHIARA CAPRIOTTI, MASSIMILIANO ANDREAZZOLI, GIUSEPPINA BARSACCHI
ggiudetti@biologia.unipi.it

Eye development is a multistep process that requires specific inductive signals and precise morphogenetic movements during embryogenesis, starting in a well-definite region of the anterior neural plate, the eye field. Recently, it was demonstrated that an elaborate gene network of eye field

transcription factors (EFTFs) contributes to specify the neural and retinal fate of the eye field. Among these EFTFs, *Xrx1* is involved in proliferation and neurogenesis in the eye field and is necessary for the correct development of the retina .
To identify genes regulated by Xrx1, we performed a high throughput screen based on Affymetrix microarrays. Our strategy consists in selecting genes whose expression level is significantly and coherently affected in both gain- and loss-of-function experiments at neurula stage.
The obtained data revealed 793 differentially expressed transcripts for the gain of function experiment and 1893 differentially expressed transcripts for the loss of function experiment. 122 transcripts were present in both gene lists.
A complete reannotation was performed for each transcript via a newly developed algorithm: analysis of the resulting datalists showed that more than 30% of the identified transcripts was poorly or not annotated.
An initial exploration of annotated transcripts in the in the gain of function gene list showed several transcripts involved in patterning, cell proliferation and cell signaling. Interestingly, several genes involved in endomesoderm development appear to be repressed by Xrx1 indicating that this transcription factor might be involved in preventing eye field cells from adopting an endomesodermal fate
Attention was focused as well on non-annotated genes whose expression was coherently affected in the experiments (i.e. genes activated in gain of function and repressed in loss of function experiments, and vice-versa). *in situ* hybridization experiments on embryos at different developmental stages (13,16,19,27,33/34, and tadpoles) were performed as a first validation of the analysis results, providing confirmations for the consistency of the microarray data: many ESTs resulted actually transcribed within the *Xrx1* expression domain, including the eye, eye-field or diencephalic structures.
Present work involves real time PCR validation, testing by *in situ* hybridization the ability of selected transcripts to respond to *Xrx1* overexpression, as well as identity and molecular function prediction by bioinformatic approach.

Screening for molecular interactors for XOTX2 and XOTX5b transcription factors.

PAMELA MANCINI[1], GIUSEPPINA BARSACCHI[1], ALVARO GALLI[2] E ROBERT VIGNALI[1]
[1]Dipartimento di Biologia, Università di Pisa; [2]Istituto di Fisiologia Clinica, CNR, Area della Ricerca, Pisa
rvignali@biologia.unipi.it

We have recently shown that XOTX2 and XOTX5b transcription factors, play crucial roles in the cell fate determination events in *Xenopus* eye, where they respectively promote bipolar and photoreceptor cell fates. The molecular basis of their differential action is not completely understood, though we were able to identify a small stretch of about 8-10 aminoacid residues (a "retinal specificity box") that makes the biological action of these two proteins completely different both in *in vivo* transfected retinal precursors and in *in vitro* transfected cell cultures. Because other molecular partners often integrate the action of homeodomain transcription factors we have started a screening for protein interactors of XOTX2 and XOTX5b, using the two hybrid screen procedure. In a first round of screening we focused our attention on potential interactors for XOTX5b. About 200 putative positive clones were isolated and are currently under characterization by direct sequencing and subsequent search in database.
Relatively little is known on what gives developmental specificity to homeodomain regulators.
We hope that results of the current research may provide hints on some of the molecular players that provide XOTX2 and XOTX5b with their appropriate developmental specificity in retinal histogenesis.

Searching for a role of protein tyrosine nitration during neuronal differentiation.

GRAZIELLA CAPPELLETTI[1], CRISTINA RONCHI[2], DANIELE CARTELLI[1], GABRIELLA TEDESCHI[2], SIMONA NONNIS[2], ARIANNA TOSCANO[1], AND ERMINIO GIAVINI[1]
graziella.cappelletti@unimi.it
[1] Dipartimento di Biologia, Università degli Studi di Milano; [2] D.I.P.A.V., Sezione di Biochimica, Università degli Studi di Milano.

Among the myriad of cellular functions played by nitric oxide (NO) in the brain, there is increasing evidence that NO might be a primary player in the programme of neurogenesis and neuronal differentiation. The signalling pathway triggered by NO in physiological processes involves the activation of soluble guanylate cyclase, S-nitrosylation of proteins, and, as recently proposed, nitration of tyrosine residues in proteins. We have previously showed that the cytoskeleton becomes the main cellular fraction containing nitrotyrosinated proteins during nerve growth factor (NGF)-induced differentiation of cultured cells. On the ground that cytoskeleton is crucial in neuronal morphogenesis to determine axonal guidance and branching, here we have investigated the possible role of nitration in controlling cytoskeleton remodeling, in particular microtubule rearrangement and dynamics, that underlines neuronal differentiation.
We have initially studied the association of nitrated proteins with the cytoskeletal fraction in differentiating neuronal cells following exposure to microtubule depolymerising treatments. Through morphological and biochemical approaches we found that nitration correlates with the increased microtubule stability underlying the progression of neuronal differentiation. Given that α-tubulin is one of the major target of nitration, we have undertaken an *in vitro* study to selectively nitrate purified tubulin and to asses the role of this post-translational modification in modulating tubulin assembly and stability. We found that modified tubulin remains competent to assembly and that nitrated microtubules show an increased stability to depolymerising agents. Finally, we are trying to modulate the level of nitrated proteins in differentiating cells and study the effects on microtubule stability and dynamics by live cell imaging.
We conclude that nitration of cytoskeletal proteins could play a novel functional role in the complex and dynamic organisation of the cytoskeleton underlying neuritogenesis and differentiation.

Differentiation of olfactory epithelium in the elasmobranch *Raja clavata*

SARA FERRANDO[1], MASSIMILIANO BOTTARO[1,2], TIZIANA FERRANDO[1], CHIARA GAMBARDELLA[3], GRAZIA TAGLIAFIERRO[1]
sara.ferrando@unige.it
[1] LIBiOM – Dipartimento di Biologia – Università di Genova [2] ICRAM c/o Museo Nazionale dell'Antartide – Università di Genova [3] – Dipartimento di Biologia Animale ed Ecologia Marina – Università di Messina

The embryonic development of oviparous elasmobranch lasts generally several weeks. At about one half of this period, some respiratory canals in the egg case become unplugged, allowing the embryo to have a better oxygenation and keep contact with the external environment. At that time, embryo needs to be able to maintain its homeostasis and, if its chemical-sense organs are working, it can receive information from the environment. In order to study the differentiation of the olfactory epithelium in cartilaginous fishes, *Raja clavata* embryos (5, 8, 13, 15 and 19 week after egg laying) and juveniles (2, 8 and 24 weeks after hatching) were considered. The embryonic development of *R. clavata*, at 15°C of temperature, lasts 24 weeks and the opening of the respiratory canals occurs after 10 weeks from egg laying. Samples were fixed in paraformaldehyde, paraffin embedded and 5 µm sectioned. Histological and histochemical stainings were performed for the morphological characterization, while immunohistochemical methods were used to highlight functional markers. The olfactory lamellae were present at 13 weeks after egg laying and the main cellular types (olfactory receptor neurons,

crypt neurons and supporting cells) became morphologically distinct at 15 weeks after egg laying. Olfactory neurons became mature, showing immunoreactivity for Olfactory Marker Protein, at 19 weeks after egg laying. At this stage, ion exchanging cells appeared along the olfactory lamellae; their activity was demonstrated by the presence of immunoreactivity for Na^+/K^+ATPase along the basolateral membrane. Thus, the olfactory epithelium of *R. clavata* is probably functional about one month before the hatching, allowing the embryo to receive chemical stimuli from the environment by the respiratory canals. In some bony fishes, the olfactory epithelium is involved in imprinting processes during the embryonic development and the very early stages of life but, to date, no data are present in literature about an olfactory imprinting in elasmobranches.

Salicylate attenuates gentamicin-induced ototoxicity in the papilla basilaris of the lizard *Podarcis sicula*

BICE AVALLONE, FABIO SASSO, GIUSEPPE BALSAMO E FRANCESCO MARMO
bice.avallone@unina.it
Dipartimento. Delle Scienze Biologiche, sez. Genetica e biologia Molecolare, Università di Napoli "FedericoII"

It's known that ototoxic side effects of gentamicin are caused by an iron-gentamicin complex that produces free radicals. Antioxidants attenuate gentamicin-induced free radical formation. We have previously demonstrated hair cell regeneration in both the crista ampullaris (organ involved in dynamic balance) and the saccule (organ involved in static balance) of gentamicin-treated *P. sicula*. We now examine the fate of papilla basilaris (organ involved in hearing) hair cells after exposure to three doses of gentamicin. We want to determine whether there is any recovery and whether this is due to repair of damaged hair cells, to regeneration or, most likely, to both. In addition, we investigate whether antioxidants can effectively inhibit gentamicin-induced hair cell loss by SEM analysis using salicylate to protect against gentamicin ototoxic effects. Moreover, using BrdU, we tested whether or not there is hair cell regeneration following administration of gentamicin. A BrdU immuno-fluorescence protocol was followed to detect BrdU-labelled cells. It was found that Gentamicin induced damage in the hair cells. Three days after the end of gentamicin treatment the hair cells of papilla showed fused or disarrayed stereovilli and there was widespread loss of hair cell bundles from the hair cells. Subsequently, numerous hair cells were lost. Conversely, when salicylate was co-administered with gentamicin the damage was moderated. The results suggest that administration of salicylate facilitates the recovery and reduces damage to hair cells after gentamicin treatment. Finally, we found evidence of bromodeoxyuridine incorporation initially in supporting cell nuclei and then in hair cell nuclei. This indicates that a process of sensory epithelium repair and hair cell regeneration occurred and that the recovery was due to both the proliferation of supporting cells and, as seems likely, self-repair of hair cell bundles in weakly damaged sensory cells.

Peroxisomes in brain aging. A morphological and biochemical study in mouse neocortex and hippocampus

SANDRA MORENO[1], ANNAMARIA CIMINI[2], LOREDANA CRISTIANO[2], FEDERICA GIARDI[2], BARBARA D'ANGELO[2], PAOLO CARRARA[1], MARCELLO D'AMELIO[3], MARIA PAOLA CERÙ[2]
smoreno@uniroma3.it
[1]Dip. di Biologia - Università "Roma Tre"
[2]Dip. di Biologia di Base ed Applicata – Università de L'Aquila
[3]IRCCS Fondazione Santa Lucia, Roma

Peroxisomes are ubiquitous cytoplasmic organelles, centrally involved in reactive oxygen species (ROS) and lipid metabolism. In the brain, their presence has been documented both in glial and neuronal cells, where they play crucial roles during development and in the homeostasis of healthy adult tissue. In cellular aging, peroxisomal functions are reportedly altered,

concomitantly with increased oxidative stress and inflammation.
The aim of this work has been to characterize the peroxisomal population in young, adult, and aging mouse brain by means of morphological and biochemical techniques. Specifically, we investigated the expression and immunolocalization of several peroxisomal markers, namely catalase, acyl-CoA oxidase, thiolase, peroxisomal membrane protein 70 (PMP70), in 3-, 6-, 9-, and 18-month-old mice, focussing on brain areas known to be vulnerable to oxidative stress, i.e., neocortex and hippocampal formation. Moreover, possible age-related variations in peroxisome-related proteins, including ROS scavenging enzymes (superoxide dismutase 1, or SOD1 and glutathione peroxidase 1, or GPX1), and proteins involved in peroxisome biogenesis (Pex5p and Pex14p) were also studied. Finally, neuronal and glial markers (NeuN and GFAP) have been used for cytoarchitectural analysis of the aging brain.
Our results demonstrate quantitative variations in the size of peroxisomal population and their protein content during aging, some functions being more affected than others. Indeed, a slight decrease between 3 and 6 months is detected for most proteins, followed by a remarkable increase at 9 months. In 18-month-old brain, when gliosis takes on, some proteins (Pex14p and PMP70) are decreased, while others (catalase and SOD1) are even more increased. Interestingly, several proteins (PMP70, peroxins, catalase, thiolase, SOD1) show age-dependent modifications in their intracellular localization, including nuclear translocation and/or retraction from cell processes to the perikaryon.
These results suggest that, in the aging brain, peroxisomes are first induced as an early response to increased ROS generation, thus contributing to cell antioxidant defence. The nuclear localization of some ROS scavenging enzymes may indicate protection of chromatin against oxidative damage. As aging proceeds (18 months), however, peroxisomal biogenesis is impaired, probably due to their membrane oxidative damage, while antioxidant enzymes further increase in both neuronal and glial cells.

The research of the genetics and neuronal basis of brain symbolic representation.

PIERO A. BATTAGLIA E FRANCA GIGLIANI
p.a.battaglia@tiscali.it

The first appearance of explicitly symbolic objects in the archaeological record marks a fundamental stage in the emergence of modern social behaviour in *Homo*.
Does exists a animal model to understand which are the developmental basis of brain symbolic representation?
The honey bee dance language presents the most elaborate recruitment among social insect and is considered the only symbolic communication used by invertebrates.
These findings raise questions about the process of encoding and decoding symbolic information in the honey bee brain.
At present two are the hypotheses proposed.
The first: memories of topographical features might be involved in dance communication.
This hypothesis assumes the existence of a "functional" map based on the information available on-site.
The second hypothesis assumes that the evolution dance language consideration is likely based on the modification of central neural pathway associated with path integration: the capability to calculate distance and directional information during flight.
In both cases the genes that determine the central nervous system development of honey bee, have an essential importance in understanding the brain symbolic representation.
Homeobox genes are required for the development of specific brain segment in honey bee and the regionalised expression of their homologs in vertebrae brain suggests an evolutionarily conserved program for brain development.
Both in insect and mouse, homologs of *Pax6* genes are expressed in and required for the development of the neural structures that are important to the learning raises the possibility

that these structure arose very early in brain evolution.
This conservation of developmental control gene action in embryonic brain patterning contrasts dramatically with the diversity of brain structure that have arisen in evolution. One solution to this apparent paradox might be that conserved genes such as the *otd/Otx* genes acquired different roles even while retaining an evolutionary functional as is the case of *Hedgehog* pathway that plays an essential role in the Diptera wing formation. The same pathway is recruited in the developing butterfly eyespots.
In conclusion, the insect brain may be a suitable model to understand the development of neuronal and genetic basis of the symbolic brain representation building..

XNOA 36 and spectrin mRNAs are located in half of Xenopus oocyte bisecting the mitochondrial cloud

MARIACARMELA VACCARO, FRANCO GRAZIANI*, SILVIA GIGLIOTTI,* ROSA CAROTENUTO, CRISTINA DE ANGELIS, CHIARA CAMPANELLA
Department of Structural and Functional Biology, Universtiy of Napoli "Federico II", Italy, *IGB, CNR Napoli, Italy.
carmenvr@inwind.it
chiara.campanella@unina.it

In recent years, mechanisms underlying primary axis determination have been uncovered in *Xenopus laevis* oogenesis (see King et al., 2005). The position of the mitochondrial cloud (MC) in st.I oocytes, determine the future vegetal pole. A major point in the study of symmetry determination in *Xenopus* is whether, in early stages of oogenesis, molecular signalling exists interacting with the MC.
In this work we have isolated a cDNA encoding XNOA36 a highly conserved zinc-finger protein, whose function is in so far unknown. Analysis of the transcript localization through in situ hybridization, coupled with immunolocalization of cytoskeletal protein were performed. These analyses were carried out both in untreated oocytes and in oocytes treated with drugs destroying the cytoskeleton. In *Xenopus laevis*, the mRNA, is expressed all along oogenesis, more abundantly in stage I oocytes than in later stages. The riboprobe decorates a filamentous network. It first distributes in the whole cytoplasm, then, in oocyte of 180-200 µm, it assumes a polarized distribution. The highest labelled region is a sector of the oocyte periphery from where the mRNA distributes with a gradient pattern along an heavily labelled network. In oocyte of 250 µm this distribution is similar to that of spectrin mRNA and is restricted to half of the oocytes, as observed in sections of the oocytes parallel or coinciding with the virtual A/V axis with the MC bisected by the riboprobes. The intriguing possibility exists that these mRNAs define a new symmetry in st I oocytes. The XNOA36 mRNA localization can be uncoupled from the spectrin mRNA trough cytochalasin B or ice-nocodazol treatment. However XNOA36 mRNA localizes with the protein spectrin, leading to conclude that messenger localizes in the oocyte through a cytoskeletal network containing spectrin

Hovering between life and death: the blastogenetic cycle of the colonial ascidian *Botryllus schlosseri*

LORIANO BALLARIN, ADAMS MENIN
loriano.ballarin@unipd.it
Dipartimento di Biologia, Università di Padova

The colonial ascidian *Botryllus schlosseri* forms new zooids by blastogenesis, through the formation of palleal buds which progressively grow and mature until an adult is formed. At a temperature of 19°C, adult zooids remain active for about one week; then they contract, close their siphons and are gradually resorbed, being replaced by buds which reach functional maturity, open their siphons and begin their filtering activity as adult zooids. This recurrent generation change, known as regression or take-over, is characterised by the occurrence of diffuse programmed cell death by apoptosis, as indicated by the increasing activity of various

caspases. In addition, the pattern of expression of Bcl-2, Bax, Fas and FasL also changes with respect to mid-cycle stages, suggesting the involvement of both the extrinsic and the intrinsic pathway in natural apoptosis of *Botryllus*.
During the take-over, colonies do not eat, but morphogenetic processes continue. The death of adult zooids puts a quantity of material at the colony disposal. Circulating phagocytes engulf apoptotic cells: in this way, nutrients derived from the demolition of adult tissues are recycled and used for the development of new tissues allowing both bud growing to maturity and haemocyte proliferation to replace senescent blood cells. Therefore, recurrent apoptosis in *Botryllus* appear as an efficient strategy for a cyclical rejuvenation of the colony involving a cross-talk between old tissues, phagocytes and developing buds and tissues.

Spermiophagic activity in the male genital tracts of Orthoptera Acrididae and Tettigoniidae

RENATA VISCUSO, MARIA VIOLETTA BRUNDO, LORENZO SOTTILE
mvbrundo@unict.it
Dipartimento di Biologia Animale "Marcello La Greca", Università di Catania

It is well known that the spermiduct and the seminal vesicles are involved in the elaboration of secretoty products that can keep the male gametes alive until they're transferred to the female genital tracts.
Nevertheless, recent studies have shown that these structures can have other specific roles within the reproductive process, that can be different, depending on the used reproductive strategy (Cantacuzène, 1971; Couche & Gillot, 1988; Tikku and Saxena, 1990; Cattaneo *et al*., 1997; Viscuso *et al*., 1999;).
On the basis of these results we have found interesting the study of such formations, taking into account especially their involvement in the processes of selection and control of the gametes that pass through them.
So we have focused our attention on study models regarding a number of species of Orthoptera Acrididae and Tettigoniidae, for which nothing is known in literature.
The results obtained have shown that for all the species considered the spermiduct can be divided into two different sections, which are called intratesticular tubule and vas deferens; the last one, before the exit into the ejaculatory duct, enlarges in order to produce the seminal vesicles.
As to the ultrastructural aspect, the most important characteristic which has been noticed in all the examined species, is the presence of a spermiophagic activity; such an activity is confirmed by the formation, within the cytoplasm, of phagosomes, containing sperms and spermatodesms, at various degradation stages; this activity could be aimed at controlling both the *quality* and *quantity* of the sperms to be sent to the female genital tracts, maybe selecting those more suitable for interaction with the female gamete.
This activity, in the species similar to Tettigoniidae, seems to exist only at the level of the epithelium of the seminal vesicles; in fact, within the vas deferens, the secretory activity could be mainly useful for the reorganisation of the spermatodesms "cap" (Viscuso *et al*., 1998; 2007).
On the other hand in the species belonging to the Acrididae, the spermatodesms cap is not greatly modified within the spermiduct (Cantacuzène, 1974; Sottile *et al*., 2003), so the spermiophagic activity takes place in the internal part of the lumen of the spermiduct, through free cells which are present in this location and whose genesis is still to be defined.

Cadmium and manganese effects on sea urchin embryo development

ANNALISA PINSINO, MARIA AGNELLO, SIMONE FILOSTO, LIANA BOSCO AND MARIA CARMELA ROCCHERI mariroc@unipa.it
Dipartimento di Biologia Cellulare e dello Sviluppo "A.Monroy", Università degli Studi di Palermo

Among compounds metals are the major contaminants generated by human activities

and represent an actual hazard for aquatic ecosystems. Marine organisms can take up metals from solution and diet. Heavy metals like Cd are toxic as trace elements, others like Mn play roles as nutrients but can act as toxicants at elevated concentrations. Stress response is stage specific and echinoid plutei and larvae are the most sensitive to pollutants.
In the present study we investigated both the distinct and simultaneous effects of exposure to Cd and Mn in sea urchin *P. lividus* embryos and their capacity to withstand to each insult, activating different strategy for protection.
We previously demonstrated that Cd exposure causes delay and morphological aberrations during sea urchin development, accompanied by the synthesis of specific stress proteins. Atomic Absorption Spectrometry (AAS) analysis pointed out the progressive accumulation of the metal in embryo cells, causing apoptosis, even reducing Cd down to environmentally-relevant concentrations. Here we demonstrate that reactive oxygen species (ROS) increase significantly during Cd insult. Moreover, we show the interference between Cd and Ca uptake during early development, suggesting the competition for using the same ionic channels.
Likewise, Mn showed inhibitory effects on embryo development, producing specific malformation in a time- and dose-dependent manner, in particular we found retarded and malformed plutei with inhibition of skeletogenesis. By AAS analysis we found that: Mn is accumulated into the embryos 24 hrs after fertilization, on the contrary, Ca concentration is reduced. Mn/Ca interaction could explains the inhibition of spicule formation from gastrula to pluteus stage and perturbation to PMC migration into the blastocoel. Conversely, we found Mn exposure does not induce Hsps neosynthesis but cause an increase in some Hsc levels. Moreover, we found that Mn exposure neither triggers apoptosis nor starts ROS production.
To study the effects of Cd/Mn co-treatment on sea urchin development, we treated the embryos, in continuous after fertilization, with a Mn concentration 10-fold molar excess with respect to Cd concentration and we observed several abnormalities and developmental delay. Cd/Mn co-treatment causes modification in terms of kinetic of protein synthesis: decrease of Hsp70/72 and increase of a 40 kDa protein synthesis.

Astenozoospermy and mitochondrial DNA

ELENA CARRA , BARBARA SALERNO, ELIANA GERARDI AND A.M. RINALDI
Dipartimento di Biologia Cellulare e dello Sviluppo "A. Monroy" Università di Palermo Viale delle Scienze, Palermo, Italy;
elecarra@unipa.it

Male infertility, inability to fecundate the oocyte, is due to alterations of sperm characteristics (low number, low motility, altered morphology), to the quantity of semen or to the presence of alterations in the male genital line.
75% of infertile men deal with untreatable sub-fertility. These subjects are either oligospermic producing less than the normal number (20 million sperm/ml) of sperms, or astenozoospermic with high percentage of immotile sperms. It is almost universally recognized that mitochondria play a crucial role in sperm health and fertility, in fact deletions or multiple rearrangements of mtDNA can cause defective sperm function (Etienne et al, 1997, 2001; St. John et coll. 2001).
Motility, viability, concentration and mitochondrial respiratory activity "*in vivo*" of semen of 100 idiopathic astenozoospermic patients and individuals with normal seminal parameters as control, were analyzed. DNAs purified form motile and immotile fractions of the same patients were subjected to conventional PCR. Results showed that mtDNA and Y chromosome regions from the motile fractions of patients and controls gave amplifications, while, the non-motile fractions of the same seminal samples did not give amplifications of ND5, ND6 and regions inside the D-loop (Carra E. et al.2002, 2004). We recently submitted the DNA of the same patients to 10 different PCR assays using primers covering 25 mtDNA loci to

amplification of short and long DNA regions. The locus analyzed comprised the following genes: for ND6, ND5, ND4 and ND4L of the complex I; COI, COII and COIII of the complex IV; ATPase 6 and ATPase 8 of complex V. The results show that only for 14 patients exhibited the predicted PCR products. *F*or the remaining 36 patients we found deletions in the genes coding for the subunits COI, COII, ND5 and ND6 and for the cytochrome b (CYTb). In addition, using primers for the region comprising the genes for ND6, tRNA Glu and the CYTb we found deletions in the mtDNA of 42 patients over 50, suggesting that the region analyzed was more prone to deletion incidence. We then related the deletions to sperm motility percentage, in order to contribute to a more precise and complete diagnosis of male infertility.

The expression of PACAP and PACAP receptors during spermatogenesis of cartilaginous fish *Torpedo marmorata*

MARINA PRISCO[1], SALVATORE VALIANTE[1], ELISA APICELLA[1], MARISA AGNESE[1], GIUSEPPINA DEL GIUDICE[1], FRANCESCO ANGELINI[2], VINCENZA LAFORGIA[1], PIERO ANDREUCCETTI[1]
[1]Department of Biological Sciences, Evolutionary and Comparative Biology Division, University of Naples Federico II, Naples, Italy; [2]Department of Structural and Functional Biology, University of Naples Federico II, Naples, Italy
piero.andreuccetti@unina.it

Pituitary adenylate cyclase-activating polypeptide (PACAP), that is a member of the secretin-glucagon peptide family, has been located in a wide range of tissue (for review see Sherwood et al, 2000; Vaudry et al, 2000), including testis, where it seems to be involved in the control of steroidogenesis (El-Gehani, 2000; Li et al, 2004; Funashabi, 2004; Lacombe et al., 2006). The aim of the present investigation is to study the distribution and the expression of PACAP and its receptors, PAC_1, $VPAC_1$ and $VPAC_2$, in the testis of a cartilaginous fish, *Torpedo marmorata*. Utilizing western blotting, immunocytochemistry and *in situ* hybridization, we showed that PACAP was highly expressed in *Torpedo* testis as well as PAC_1 and $VPAC_2$, while $VPAC_1$ was found to a lesser extent. Particularly, PACAP and its receptors were expressed in those cells actively involved in testicular steroidogenesis, as prespermatogonia, Leydig and Sertoli cells. Moreover, we showed that PACAP and its receptors were also expressed in spermatids and spermatozoa, i.e. during spermiogenesis , thus suggesting that PACAP has a role not only in steroidogenesis but also in spermiogenesis of cartilaginous fish *Torpedo marmorata*.

Analysis of the distribution of Vasa protein during the early oogenesis of the lizard *Podarcis sicula*

MARIA GABRIELLA MAURIZII*, VALERIA CAVALIERE*, CHIARA GAMBERI**, PAUL LASKO** GIUSEPPE GARGIULO*, CARLO TADDEI*
carlo.taddei@unibo.it
*Dipartimento di Biologia Ev. Sper. Università degli Studi di Bologna.
**Department of Biology, McGill University, Montreal, Québec, Canada.

In all animals information is passed from parent to offspring via the germline, which segregates from the soma early in development and undergoes a complex developmental program to give rise to the adult gametes. Comparative analysis of a number of diverse animals, including both invertebrates and vertebrates, has highlighted several remarkable features of germline development. Germline determination for many animals relies on the localization of germline factors. Germ cell differentiation also requires complex mechanisms of gene regulation, involving both transcriptional and translational control.
Among the molecules identified in *Drosophila* as being required for germ line establishment and germ cell development, Vasa is especially well conserved in evolution. *vasa* homologues have been

identified in *C. elegans*, *Xenopus*, mouse, rat, zebrafish, chicken and human.
We have started the analysis of Vasa protein in the ovarian follicle of the lizard *Podarcis sicula*. The interesting feature of the lizard oogenesis consists in the structural and functional integration, through intercellular bridges, of peculiar follicle cells, the pyriform cells, with the oocyte during most of its previtellogenic growth. These cells resemble in their functions the *Drosophila* nurse cells, but differently from these the pyriform cells have a somatic origin.
By immunoblotting and immunocytochemical techniques we found that the Vasa protein is expressed in the gemline of *Podarcis*.
We will present confocal data showing the specific localization of Vasa protein during the early steps of *Podarcis* oogenesis.

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Somatic embryogenesis in *Cyclamen persicum* Mill.

M. SAVONA*,G. FALASCA°,B. RUFFONI*,M. TROVATO°°,S. DE VRIES^, M. M. ALTAMURA°
*CRA-FSO Unità di Ricerca per la Floricoltura e le Specie Ornamentali; ° Dip. Biologia Vegetale, Sapienza Università di Roma; °° Dip. Genetica e Biologia Molecolare, Sapienza Università di Roma; ^ Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands.
mariamaddalena.altamura@uniroma1.it

Cyclamen persicum Mill. is an ornamental species with a great economic importance, and is mass-propagated exclusively via F1 hybrid seeds. At present, a biotechnological goal in this plant is to set up a rapid and efficient system for obtaining artificial seeds for reducing the production costs and for increasing the commercial yield of selected genotypes superior for ornamental characters. We studied the *in vitro* propagation potential of *Cyclamen persicum* Mill. cv. 'Halios' via somatic embryogenesis, i.e., via the production of embryos starting from immature ovaries cultured on a selected medium (Schwenkel and Winkelmann, 1998). Our aim was to apply this culture system to different clonal lines of horticulture importance in order to investigate the type of the embryogenic process, to select specific marker genes for the somatic embryo formation, and to characterize cyto-histologically all the phases of embryogenesis for identifying the best embryo stage to be encapsulated to form low cost artificial seeds. The histological analysis showed that the somatic embryos were always generated after callus formation, and started from proembryogenic masses in the callus (indirect genesis). The explant juvenility was confirmed to be important for inducing pro-embryogenic masses. It was also observed a clonal behaviour in the callus lines obtained: the calli showed different colour and compactness and also a different embryogenic potential. The somatic embryos of all the embryogenic genotypes were able to mature, to germinate and to develop into complete plantlets, which were then transferred *ex vitro*. In order to evaluate the homogeneity of the crop, i.e. for excluding somaclonal variation events which might be possible due to the indirect origin of the embryos, morphological features as plant height, leaf length, open flower height and petal number were measured. The results showed a high phenotypic homogeneity of the somatic embryo-derived plants that can be considered "true-to-type plants". Furthermore, a comparative analysis of cyclamen somatic and zygotic mature embryos by proteomics (Winkelmann *et al.*, 2006) confirmed our results about the good quality of the somatic embryos in comparison to the zygotic ones, sustaining their possible use in artificial seeds. The histo-anatomical phases that led from the callus to the neoformation of the somatic embryos were identified, and the presence of organogenic events (i.e. *de novo* formation of shoots and roots in the callus), which were competitive to somatic embryogenesis, were detected in specific genotypes.

In cyclamen, a multigene family of *SERK* genes *(Somatic Embryogenesis Receptor Kinase)* was identified by us. One of the obtained sequences showed a high identity percentage with *AtSERK1*, a gene that is considered a marker of the early events of somatic embryogenesis both in *Arabidopsis* and in some other species (Albrecht et al., 2005, and references therein). Another sequence showed identity with *AtSERK3 (BAK1)*, a gene that has an important role in brassinosteroid signal transduction, and that exhibits a central role in innate immunity in plants (Heese *et al.*, 2007, and references therein).
Preliminary whole-mount *in situ* hybridization with digoxigenin (DIG)-labeled cyclamen *SERK* RNA probe revealed expression in all cell populations with meristematic features (i.e. embryonic tissues, and meristematic apices of shoots and roots). The absence of the hybridization signal in the non embryogenic calli, and the high signal combined with the low frequency of organogenic events in the high embryogenic genotypes, support our hypothesis that *SERK* could be a strong marker of the induction of the embryogenic competence in cyclamen. The possible different roles of the various members of the gene family in embryogenesis *vs* organogenesis is under study.
Furthermore, the appropriate nutritional compounds of artificial secondary endosperm was determined, and the incapsulated somatic embryos were obtained. These artificial seeds were able to germinate into plantlets, showing that the final goal of the project was reached.

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Effects of rhizospheric microrganisms on root system development, in a number of experimental systems

GRAZIELLA BERTA, ELISA GAMALERO, GUIDO LINGUA E ANNA FUSCONI
Graziella.Berta@mfn.unipmn.it

Plant roots interact with soil-born microorganisms, such as beneficial or deleterious bacteria and fungi. Several beneficial microorganisms are mutualistic symbionts: among them, arbuscular mycorrhizal (AM) fungi colonize the root tissues of most terrestrial plants. AM fungi develop intercellular hyphae and form, after penetrating the cell wall, a specialized and branched structure, called the arbuscule, surrounded by the plant cell membrane. This is the site where nutrient exchanges occur: of photosynthates, from the plant to the fungus, and minerals, mainly phosphate, from the fungus to the plant. In addition to AM fungi, also rhizobacteria, free-living microorganisms inhabiting the volume of soil surrounding the root systems, are present.
It is well known that both AM fungi and some rhizospheric bacteria (called Plant Growth-Promoting Rhizobacteria - PGPR) can positively affect plant growth, improve plant health and productivity and affect root morphogenesis. Root system development is indeed highly variable, and root plasticity allows plants to optimize the acquisition of resources from the soil. Interactions with soil microorganisms and variations of the

concentration of nutrients in the growth medium may deeply modify the plan of root development. AM generally increase the total root length and, sometimes, the mean diameter of the roots, the second one related to the larger diameter of the root apices. However, the most frequent effect of AM is an increase in the number of lateral roots, which gives rise to a more branched root system. Anyway, the order of the roots involved varies: in the monocot *Allium porrum* the increase of branching occurs in adventitious roots. On the contrary, the degree of branching of the axes of the woody dicotiledonous plants *Prunus cerasifera* and *Platanus acerifolia* is almost unaltered. The largest morphological effect of AM colonization is the increased branching of first order lateral roots in the former and of the development of laterals of higher order in the latter.

Variations in root system morphology are mainly related to a different behaviour of their apices. In *A. porrum* plants AM causes a reduction of the percentage of mitotically active apices and mitotic cycles become longer with increasing colonization levels. Alterations in lateral root development are especially evident in a maize mutant lacking of laterals on the primary and seed roots (*lrt1*), where the establishment of symbiosis with *G. mosseae* is associated with the occurrence of a new type of lateral root stunted and highly branched. AM increase phosphate (P) absorption from the soil, and it is well-known that P is a key regulator of root morphogenesis. Unfortunately, the mechanisms of the morphogenetic responses of the root to P availability have been studied mostly in *Arabidopsis thaliana*, a non-mycorrhizal plant. *A. thaliana* responds to low phosphate with a reduction of the growth of the primary root and an increase in lateral root number and length with a determinate pattern of growth. If the responses of *A. thaliana* could be extended to other plant species, the establishment of AM symbiosis, causing an increase of P concentration in the plant tissues, should reduce root branching, instead of stimulating it, but this is not the case. On the other hand, increases of P concentration leads to a higher number of adventitious roots and of their branching. Such differences probably depend on the different nutritional strategies adopted by mycorrhiza-forming, or not, plants. Further studies, running at present in our laboratories, are necessary in order to gain further knowledge on the relationship between nutritional strategies and morphogenesis in model plants able to form mycorrhizas.

The pathways controlling morphogenetic responses in the root systems of AM plants are still known rather poorly. It has recently been shown that treatments with either Nod factors or with a diffusible product of AM fungi stimulate the formation of lateral roots, while this does not occur following the supplementing of auxins, one of the main hormones involved in the formation of lateral roots. Nevertheless, several papers have reported higher hormone concentrations in mycorrhizal roots, with possible effects on root branching. In additions, several microrganisms interacting with plant roots can produce and release plant-hormone analogues. Therefore, symbiotic associations might use an hormonal pathway to control root branching.

The impact of rhizobacteria on root morphogenesis has been studied starting from the beginning of 2000, but knowledge on the mechanisms involved is still scanty. The first paper published on this topic described the impact of *Pseudomonas fluorescens* A6RI on root morphogenesis of tomato grown under different fertility conditions. Plants inoculated with the beneficial rhizobacteria showed increased biomass as well as longer root, with higher surface area and volume compared to untreated controls. Nevertheless, the positive impact of the bacterial strain occurred only in plants cultivated under high fertility conditions.

Although the increase of total root length in plants treated with *P. fluorescens* A6RI could be related to the synthesis of indoleacetic acid (IAA), the mechanisms involved in plant growth promotion by rhizobacteria are strictly dependent on the strain and on the plant type and could change according to the

environmental conditions and are therefore difficult to understand.

Recently, the effects of *Bacillus megaterium* were investigated *in vitro* on wild type *A. thaliana* and on various mutants defective for the auxin and ethylene signalling pathways. The inoculation of the mutant defective in the IAA synthesis lead to the inhibition of the primary root growth and to the increase of lateral root number and length as well as of the length of root hairs. All mutants showed increased biomass following bacterial inoculation; in addition *aux1-7* and *eir1* (auxin defective) and *etr1* and *ein2* (ethylene defective), characterized by a limited root hair and lateral root production, increase the number of laterals and develop long root hairs following inoculation. These data indicate that the products of the above mentioned genes are not directly involved in the observed modifications of the root systems and that, probably, the bacterium affects root growth by auxin- and ethylene-independent mechanisms.

Co-inoculation of PGPR and AM fungi could lead to synergistic effects on plant growth and root architecture. However, the mechanisms involved in synergistic interactions are still under debate and can be investigated by means of bacterial mutants, a useful tool in order to improve our understanding of the stimulation of plant growth and in the modification of root development. It has recently been shown that *Pseudomonas putida* UW4 ($AcdS^+$), able to synthesize the enzyme 1-aminocyclopropane-1-carboxylic (ACC) deaminase and to reduce ethylene level *in planta*, therefore increasing plant tolerance to stress, as well as its mutant defective in the synthesis of the enzyme ($AcdS^-$), did not affect root architecture in cucumber. Nevertheless, plants co-inoculated with the $AcdS^+$ strain and the AM fungus *Gigaspora rosea* BEG9 showed longer roots, with a high number of root tips. This positive effect was related to the increase of mycorrhizal colonization induced by the strain UW4 $AcdS^+$, but not by its mutant $AcdS^-$. These results clearly demonstrated that the enzyme ACC deaminase, although being considered as one of the main mechanisms of plant growth promotion by bacteria, did not affect root morphogenesis, but can be involved in AM fungi/plant interactions.

Different results have been obtained by exposing plants to salinity and inoculating them with the same microorganisms. In this stressful condition, both the strain UW4 $AcdS^+$ and *Gi. rosea*, inoculated alone, stimulated plant growth and lead to the development of longer roots, therefore more efficient in soil exploration, but the synergistic effects observed under optimal growth conditions disappeared.

By comparing these results with those previously described it is possible to suggest that under optimal conditions, bacterial ACC deaminase acts on the plant/AM fungus interactions, while under salt stress this enzyme is mainly involved in the plant/bacterium interactions.

Further assays based on the use of bacterial mutants producing different amounts of IAA, aiming at elucidating the role of bacterial IAA in plant growth promotion and in root development, are currently in progress both *in vitro* and *in vivo*.

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Molecular and cytophysiological events underlying ectopic morphogenesis (epiphilly) in a somaclonal variant of interspecifi hybrid *Helianthus annuus* L. x *H. tuberosus*

MARIA BEATRICE BITONTI[1], LEONARDO BRUNO[1], ADRIANA CHIAPPETTA[1], D. IARIA[1], RITA BARALDI[2], HARRY VAN ONCKELEN[3], MARCO FAMBRINI[4], VANIA MICHELOTTI[4], MARIANGELA SALVINI[3], CLAUDIO PUGLIESI[3]
[1] Dipartimento di Ecologia , Università della Calabria; [2] CNR IBIMET Sez. Bologna; [3]Department of Biology, University of Antwerp;[4] Dipartimento delle Piante Agrarie; Sezione di Genetica, Università di Pisa. b.bitonti@unical.it

The somaclonal variant of the interspecific hybrid *Helianthus annuus* x *H. tuberosus,* EMB-2*,* shows an unusual pattern of development in that it features a prominent proliferation of ectopic structures on the leaves. More precisely, in addition to leaves that expand normally (non-epiphyllous leaves, NEP), this clone produces, both *in vitro* and *in vivo*, leaves (epiphyllous leaves, EP) exhibiting embryo- and, less frequently, shoot-like structures (1). As a general rule, these ectopic structures occur on the most basal leaves and are usually arranged in clusters along veins on the proximal half of the leaf, especially at the junction between the enlarged petiole and lamina.
Embryogenesis represents a very complex developmental process, which relies on a spatially coordinated establishment of several cell identities. However, in plants embryogenesis is not strictly dependent on fertilization, because a variety of dicot and monocot species reproduces asexually by apomixis (2). Moreover, gametophytic and somatic cells can be induced by *in vitro* systems to undergo embryogenic development. Embryos can also develop from single cells of stem or leaf epidermis, a process that occurs naturally in epiphyllous species (3), as well as in somaclonal variants like EMB-2 clone (1). On the other hand, the development of new plantlets can also frequently occur through the formation of adventitious meristems on the leaves, following either developmental or environmental signals (3). Due to the capacity of plant cell to re-acquire totipotency and re-

express meristematic or embryogenic competence, higher plants exhibit an enormous morphogenetic plasticity and maintain high potential for regeneration also in *in vitro* conditions. This feature is widely exploited as a useful tool for genetic improvement of plants as well as for advanced biotechnology.

Cell totipotency/pluripotency and cell differentiation, which are essential events for plant growth and development, relies upon a strict genetic control that in the model plant *Arabidopsis thaliana* is becoming even more dissected. The emerging picture is that, both in embryonic and post-embryonic development, a key role is played by specific transcriptional regulators that interact with different hormone classes, depending in their action on internal and/or external signals. For instance, specific genes that play regulatory roles in specific embryogenesis phases or during the whole process have been identified (4-6). Notably, a direct interaction with hormone-response genes has been demonstrated for some of these transcription factors, such as the *LEC* gene family (7-10). For example, the penetrance of the *turnip* (*tnp*) mutant phenotype of *Arabidopsis*, a gain-of-function mutant of *LEC1*, is strongly enhanced or antagonized in the presence of exogenous auxin and cytokinins, respectively (11). Upstream of the *LEC* genes, *PKL*, a CHD3-chromatin remodelling factor, is necessary for their repression outside the embryo (12). In addition, members of *VP1* and *ABI3-LIKE* family of B3 domain transcription factors function as global repressors of the LEC1/B3 transcription factor network in germinating seedlings (13).

An intricate genetic network is also active in the shoot apical meristem (SAM) which is precociously determined during embryogenesis through an intricate positional signalling (14-16 and references herewithin cited). Essentially, meristem cell fate in the SAM is established and maintained by two-way signalling which involves the homeodomain transcription factors *WUS* and *STM* (14-16 and references herewithin cited). These two factors act indipendently but play complementary role. More precisely *STM* function is restricted to specify stem cells identity, while *STM* is required to prevent organ formation and mantain the SAM. An overlap between the effector genes could involve cell cycle regulation. Namely, both *STM* and *WUS* regulate genes which are involved in either cytokinin signalling or biosynthesis, that in turn can stimulate, almost in the SAM, cell division through the promotion of cyclin- D expression (14-16 and references herewithin cited). In this context we may recall that the repression of differentiation and the manteinance of cell division potential in the SAM are essential for the reiterative organ formation during plant post-embryonic development. Thus, the role of class-1 KNOX genes, which includes *STM*, is to suppress the activity of factors that promote differentiation, such as myb transcription factor *AS1*, that is in line with the role of stem cell regulators in animals (17). Furthermore, a tight link between KNOX gene expression and endogenous hormone homeostasis, involving cytokinins, gibberellins and auxin, is well-established (14-16 and references herewithin cited).

Although a more complex network underlying cell fate is progressively emerging, a complete view of the transcriptional regulators and their target genes as well as of the hierarchy of their action is not yet fully achieved. Furthermore, we are still far behind from having unravelled the mechanisms whereby a cell interprets its position, decides its fate and communicates it to the other. Similarly, how differentiated cells can reacquire either embryogenic or meristematic competence, thus producing somatic embryos and adventitious meristems, is not yet fully understood. So far, initiation of somatic embryos from vegetative cells has been verified in plants mis-expressing embryo-specific genes such as *LEC1*, *LEC2* and *HaL1L* (18-20). Furthermore there is evidence that *SERK* gene is involved in both zygotic and somatic embryo initiation (21) as well as in the development of apomictic embryo (22). Thus, zygotic, apomictic and *in vitro* embryogenesis results onto closely related pathways. However, relatively little is known about the early inductive events underlying

the transition of a somatic cell into an embryogenic pattern formation. In most cases, the supply of growth regulators or the exposure to stressful conditions is necessary for inducing embryo development (23). It is generally believed that *in vitro* embryogenesis is mediated by a signalling cascade triggered by auxin (23). In particular, in *H. annuus* it was proven that an endogenous auxin pulse is among the first signals leading to the induction of somatic embryogenesis from immature zygotic embryos (24). As for adventitious meristems are concerned, it has been demonstrated that the ectopic reactivation of KNOX genes can restore organogenic potential in transgenic plants (14-16 and references herewithin cited). Adventitious meristems have also been observed in transgenic plants with enhanced cytokinin biosynthesis (25) as well in mutants with impaired auxin transport (14-16 and references herewithin cited).

To date, most of reported evidences derive from studies on Arabidopsis thaliana plants and knowledge on other species is still very poor. In this context we performed an extensive analysis on the molecular and hormonal mechanisms related to the switch of cellular fate in EMB2 clone (20,26). At this aim, some morphogenesis-related genes, such as two class-1 KNOX genes (HtKNOT1/A2 KNOT1-1, A2 KNOT1-2) and their putative repressor AS1, as well as a LEAFY COTYLEDON1-LIKE gene (HaL1L), were identified in either the parental species H. tuberosus or in a non-epiphillous A2 clone of the interspecific hybrid. The transcription level of these genes was evaluated in both EP and NEP leaves of EMB-2 clone and in A2 leaves by eiher semiquantitative or quantitative RT-PCR and in situ hybridization. Endogenous level and spatial distribution of free indole-3-acetic acid (IAA) and citokinins were also estimated by a capillary gas-chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) method and an immuno-cytochemical approach, respectively. So far, the results obtained showed that EMB2 plants are not cytokinin-or auxin overproducers, but a localized, tissue- specific accumulation of these hormones differentially marked the morphogenic competence of epidermal cells of EP leaves. Notably, a distinct pattern of both IAA and zeatin localization was observed in the shoot-like vs embryo-like structures during their development, consistent with the differential role of auxin and cytokinins in the two morphogenetic events. A clear alteration in the expression pattern of above mentioned genes, putative marker of either embryogenic or meristematic competence, preceded the occurrence of the morphogenetic events in the leaf. In addition the transcription of HaL1L was rapidly activated in NEP leaves induced to produce ectopic embryos by in vitro culture. However, exogenous auxin treatments lightly influenced HaL1L transcript levels and somatic embryogenesis, while zeatin supply induced a massive regeneration along with a strong accumulation of HtKNOT1 (26-28). Work is in progress to verify the expression pattern of genes related to hormones synthesis/catabolism and transport. In this emerging picture it appears clear that in EP leaves a dynamic genetic network underlies the capacity of cells to resume either meristematic or embryonic fate. That is consistent with the well documented capacity of plants to re-establish stem cell niches in relation to positional cues rather then cell lineage, as evidenced following laser ablation of RAM and SAM as well during lateral root and bud formation. However, further studies are required to dissect both internal determinants and positional signalling acting at early stages of the complex epiphyllous phenomenon exhibited by EMB-2 plants.

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Transglutaminases, aspects of their enzymatic activities in plants and animals.

SERAFINI-FRACASSINI D., DELLA MEA M., IORIO R. E DEL DUCA S.
donatella.serafini@unibo.it

Transglutaminases have been studied in plants since twenty years in investigations aimed at interpreting the molecular mechanisms by which polyamines affect plant growth and differentiation. The post-translational modification of proteins by polyamines forming inter- or intra-molecular cross-links has been the main transglutaminase reaction studied in plants. Transglutaminase activity is ubiquitous: it has been detected in different organs and sub-cellular compartments, chloroplasts being the best-studied organelles. Specificity and similarity with animal transglutaminases are discussed in the light of their biochemical characteristics and functional roles. Possible roles concern the structural modification of specific proteins. They modify actin and tubulin, also influencing motor proteins, thus exerting a role on cell growth and cell structure. In chloroplasts, transglutaminases appear to stabilise the photosynthetic complexes and Rubisco, being regulated by light and other factors, and possibly exerting a positive effect on photosynthesis and photoprotection. Preliminary reports suggest an involvement in construction/organisation of the cell wall and extracellular matrix in relationship with fertilisation. Other roles appear to be related to stresses, senescence and programmed cell death. The cross recognition of substrates between plant and animal enzymes suggest both similarities and differences.
The few plant transglutaminases sequenced so far have little sequence homology with the best-known animal enzymes, except for the catalytic triad; however, they share a possible structural homology. Proofs of their catalytic activity are: 1. their ability to produce glutamyl-polyamine derivatives, 2. their recognition by animal transglutaminase antibodies, 3. biochemical features such as calcium- and GTP-dependency, etc. 4. Inhibition by specific inhibitors of animal transglutaminases. However, many of their

fundamental physiological properties still remain elusive. At present, it is not possible to classify this enzyme family in plants owing to the scarcity of information on genes encoding them.

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Spermidine inhibits cell cycle progression and induces developmental cell death in the primary root of maize seedlings.

ALESSANDRA TISI[1], RICCARDO ANGELINI[1], SANDRA MORENO[1], SERGIO LUCRETTI[2], RODOLFO FEDERICO[1] AND ALESSANDRA CONA[1]
cona@uniroma3.it
[1] Dipartimento dI Biologia, Università "Roma Tre", Viale G. Marconi 446, 00146 Roma, Italia
[2] Laboratorio di Genetica e Genomica Vegetale, ENEA, Centro Ricerca Casaccia, 00060 Roma, Italia
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Polyamines (PA) putrescine, spermidine (Spd) and spermine are ubiquitous aliphatic polycations with pleiotropic biological effects, including modulation of cell proliferation, differentiation and programmed cell death (PCD). Hydrogen peroxide (H_2O_2) deriving from oxidative catabolism of PA is a signalling mediator in inducing expression of defence genes, hypersensitive response (HR) cell death and developmental PCD. The considerable presence of polyamine oxidase protein in developing xylem tissues of *Zea mays* and the expression of a diamine oxidase encoding gene in the same tissues of *Arabidopsis thaliana* have suggested the involvement of PA catabolism in PCD occurring during differentiation of vascular tissues. In this work we present evidence that Spd supply inhibits maize root elongation and induces early differentiation of metaxylem and protoxylem vessels. To study the role played by maize polyamine oxidase (ZmPAO)-mediated H_2O_2 production in root growth and differentiation, we exploited the *in vivo* use of *N*-prenylagmatine (G3) and guazatine, two strong inhibitors of PAO activity, as well as dimethylthiourea (DMTU), a H_2O_2 scavenger. H_2O_2 deriving from Spd catabolism negatively affects root growth by inhibiting both elongation growth and cell cycle progression. UV-induced autofluorescence and laser scanning confocal microscopy analysis in cross sections of primary roots from Spd-treated maize plants reveals an intensification of autofluorescence in cell walls of rhizodermis, xylem vessels and vascular parenchyma. Flow cytometry of nuclei from untreated roots shows a % of cells at different cell cycle phases as 36.6% at G1, 34.8% at S and 28.5% at G2, while cells from Spd-treated roots shows a reduced % of S-phase cells (20.5%) in respect to control and a larger amount of cell resting into G1 and G2 phases (46.8% and 38.8%, respectively). Furthermore, Spd treatment induces DNA fragmentation throughout the root tissues as revealed by TUNEL staining. LSCP analysis of whole roots stained with propidium iodide reveals an early differentiation of metaxylem and protoxylem vessels in Spd-treated plants. Treatments with G3 or guazatine as well as with DMTU revert Spd-induced autofluorescence, DNA fragmentation and inhibition of cell cycle progression, suggesting a role for H_2O_2 deriving from polyamine catabolism as a mediator of cell death and cycle regulation.

Differential regulation of the antiproliferative gene dpc3 during growth and differentiation of D. discoideum identifies a new subpopulation of Anterior-Like Cells.

DANIELE CONTE, SILVIA ARNONE AND ADRIANO CECCARELLI
adriano.ceccarelli@unito.it

D. discoideum is an amoebozoan that grows as single amoebae feeding upon bacteria. Under starvation conditions, proliferation is

arrested and the amoebae undertake a complex differentiative and morphogenetic program at the end of which a multicellular structure comprised of a mass of spores suspended atop of a thin stalk is fomed. For its ability to undergo a multicellular stage D. discoideum has also been indicated as facoltative metazoan.
Multicellularity is acquired by aggregation, mediated by chemotactic movement, and is organized by diffusibile signals such as cAMP, and DIF. DIF is a morphogen that induces cell to differentiate into the pre-stalk/stalk pathway. In principle all cells participating to the aggregative phase have the same differentiative potential, nevertheless a strong bias for the differentiatiion into either cell type is present in each phase of the cell cycle, thus establishing a rather precise link between cell fate and the position in cell cycle at the time of starvation.
It is therefore interesting to elucidate the molecular basis of the regulatory mechanisms linking cell cycle progression to fate choice. In the search for factors involved in this mechanism we have previously characterized rblA, the ortholog of Rb (retinoblastoma) in vertebrates, and shown that it is acting at the pre-spore vs pre-stalk switch
We have now isolated and functionally characterized Dpc3, the D. discoideum homolog of the antiproliferative gene PC3 which has roles in the control of cell cycle and differentiation during vertebrate neurogenesis.
Dpc3 ORF is 1272 nt long and encodes a peptide of 423 aa with 49% homology with the human Tob1.
During vegetative growth Dpc3 gene is expressed in a cell cycle-dependent way, with a peak in mid-late G2, and it is also expressed in development when it is already detectable during aggregation. At postaggregative stages dpc3 is expressed in cells with the morphological characteristics of ALC, and in pstA cell, while at culmination ALC-specific expression is mostly switched off, resulting in a predominant stalk-specific pattern in mature culminants.
Vegetative and ALC-specific expression are dependent on expression of rblA, the D. discoideum ortholog of Rb (retinoblastoma). On the contrary, stalk-specific expression is independent of rblA, suggesting that dpc3 is controlled by different mechanisms in vegetative growth and development.
The study of the expression of several ALC specific markers in combination with Dpc3 in both wild ype and $rblA^{null}$ mutant, identifies a new population of ALC cells, differing from the known ALC in their position within the slug, as well as for their cell cycle position at time of starvation.
DDPC3 ALC-specific expression pattern is altered in DimB, a mutant in the DIF signalling pathway in which a DIF-responsive bZIP transcription factor is inactivated by insertional mutagenesis. The observation that Dpc3 expression is controlled by DIF is in agreement with the notion that $rblA^{null}$ cells are oversensitive to DIF

The cholinergic gene locus in the amphioxus *Branchiostoma floridae*

SIMONA CANDIANI, LUCA MORONTI, MARIO PESTARINO
pesta@unige.it
Department of Biology, University of Genova, viale Benedetto XV 5, 16132, Genova, Italy

The cholinergic gene locus (CGL) consists of a gene encoding for two specific cholinergic neuronal markers: the vesicular acetylcholine transporter (VAChT) and the biosynthetic enzyme for the acetylcholine synthesis, choline acetyltransferase (ChAT). VAChT is encoded by sequence in the first intron of *ChAT* and only 5' non coding exon is shared between the two transcripts. Thus, the unique gene organization of the *VAChT*/*ChAT* locus offers the possibility of coordinated regulation of two proteins required to express the cholinergic phenotype. In *Caenorhabditis* and *Drosophila*, the transcriptional regulation of CGL seems to be driven by the same transcriptional enhancer, whereas in mammals common upstream elements together with separate enhancers are present. To better

understand the regulatory mechanisms of CGL in chordates, we isolated the amphioxus homologs of *VAChT* and *ChAT* and examined their expression during development. Our results confirm that the genomic organization of the amphioxus CGL is similar to that reported from other vertebrate and invertebrate species. However, the occurrence of 5'UTR splice variants for *VAChT* suggests that, as in mammals, additional splicing regulatory mechanisms are present in the amphioxus CGL. In chordates, cholinergic transmission is typically a characteristic of motoneurons, although in vertebrates the presence of cholinergic neurons are also found in some classes of interneurons. In amphioxus, *ChAT* and *VAChT* are coexpressed in the hindbrain homologous region in several types of motoneurons and some of the interneurons. Vertebrate hindbrain segmentation includes the patterning of cranial motor neurons, which can be identified by their expression of *islet1*. From a developmental standpoint, there is a an intriguing early CGL expression pattern that is markedly similar to that of amphioxus *islet1*: in neurulae the same number of cell pairs is marked by islet and CGL, and they lie at the junctions between somites from S1/2 to S5/6. Thus, even if the amphioxus hindbrain homologous is not overtly segmented, vertebrate rhombomeres may derive from a cryptically segmented brain present in the amphioxus/vertebrate ancestor. Finally, our results show that there is indeed an initial segmental component to the early patterning of the cholinergic system in amphioxus, an aspect that needs to be considered in any explanation for the origin of hindbrain segmentation in vertebrates. (Grant from MIUR-PRIN 2006058952).

_S_-glutathionylation of metallothionein by nitrosative/oxidative stress

MARCO COLASANTI, MANUELA CASADEI, FABIO POLTICELLI, GIORGIO VENTURINI, GIOVANNI MUSCI AND TIZIANA PERSICHINI
Dip. Biologia, University "ROMA TRE", Viale Marconi 446, 00146 Rome, Italy.
colasant@uniroma3.it

Cysteines residues within metallothionein (MT) structure have been shown to be particularly prone to nitric oxide (NO)-mediated *S*-nitrosylation. In this study, the possibility that MTs undergo *S*-glutathionylation under nitrosative/oxidative stress was examined.
MT from rabbit liver was treated with different concentrations of GSNO, diamide plus GSH or H_2O_2 plus GSH. Parallel sets of samples were treated with 10 mM DTT for 30 min at 37°C to reduce mixed disulphides. Incubations were then processed for Western blot or dot-immunobinding assay. Western blot with anti-MT or anti-GSH were also performed on peripheral blood mononuclear cell extracts. Structural aspects of *S*-glutathionylation of MTs were also examined.
Treatment with GSNO, diamide/GSH or H_2O_2/GSH induced a dose-dependent increase in the levels of MT *S*-glutathionylation. This effect was completely reversed by treatment with the reducing agent DTT, indicating that *S*-glutathionylation of MT protein was related to formation of protein-mixed disulphides. Structural analysis of rat MT indicated that cysteine residues located in the N-terminal domain of the protein are the likely targets for *S*-glutathionylation, both for their solvent accessibility and electrostatics induced reactivity.
S-glutathionylation of MT, given its reversibility, would provide protection from irreversible oxidation of Cys residues, thus representing a mechanism of high potential biological relevance.

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Possible mechanisms regulating the expression of nuclear and mitochondrial genes

GIUSEPPE CANNINO, ELISA FERRUGGIA AND ANNA MARIA RINALDI
canninogiuseppe@unipa.it

In higher eukaryotes, nucleus encodes all mitochondrial proteins with the exception of

13 subunits of the respiratory complexes. These subunits, together with 2 rRNAs and 22 tRNAs, specific for the mitochondrial translation machinery, are coded by mitochondrial genome. Cytochrome *c* oxidase (COX) complex is built up with both nucleus and mitochondrion-encoded subunits. Biogenesis and assembly of the complexes thus require fine interplay between the two compartments. In order to shed light on the regulation of nuclear-mitochondrial interactions, we studied the expression of COXIII (mitochondrion-encoded) and COXIV (nucleus-encoded) subunits. We searched for RNA-binding proteins (RBPs) that could participate to the regulation of both in rat tissues.

We previously demonstrated that the expression of COXIII and COXIV subunits is different in the diverse tissues and in the developing rat brain. Since the levels of COXIV protein and mRNA are not linearly related we hypotized post-trascriptional mode of regulation. We demonstrated, by T1 nuclease protection assay, that in developing rat brain the cytoplasmic and mitochondrial fractions, contain two specific COXIV RNA-binding factors (20 and 60 kDa). The 60 kDa mitochondrial factor decreases during development with inverse correlation with accumulation of mature COXIV in the organelles. Then, we found that cytoplasmic fractions of heart, kidney and testis contain a factor able to bind the 3'-UTR of COXIV mRNA. We also found that mitochondrial extracts of brain and testis, but not of other tissues, contain a protein specifically binding COXIII mRNA. These proteins were absent in the cytoplasmic fractions of the same tissues. This may suggest the occurrence of tissue-specific post-transcriptional regulation, or post-translational modification of the involved protein.

Recently, we found that the 60 kDa factor binds only the first 38 nucleotides of 3'UTR COXIV. The function of the factor is not yet known but it could exert some role in the messenger RNA translation. At moment we are in the way to isolate, purify and characterize the factor, by streptavidin affinity chromatography methodology.

Effects of cadmium chloride on some mitochondria-related activity and gene expression of human MDA-MB231 breast tumor cells

GIUSEPPE CANNINO, ELISA FERRUGGIA, CLAUDIO LUPARELLO AND ANNA MARIA RINALDI
elisaferruggia@unipa.it

It was reported that cadmium is able to exert a cytotoxic effect on tumor MDA-MB231 cells, which shows signs of "non-classical" apoptosis and is characterized by drastic changes in gene expression pattern. In this study, we have extended our knowledge of metal-breast cancer cell interactions by analyzing some mitochondria-related aspects of the stress response to $CdCl_2$ at either 5 or 50 μM 24- or 96-h exposure, by cytochemical, conventional PCR and Northern/Western blot techniques. We demonstrated that i) no modification of the mitochondrial mass was detectable due to $CdCl_2$ exposure; ii) the respiration activity appeared to be increased after 96-h exposures, while the production of reactive oxygen species was significantly induced, as well; iii) *hsp60, hsp70, COXII* and *COXIV* expressions were dependent on the duration of Cd exposure; iv) a different hsp60 protein distribution was observed in mitochondrial and post-mitochondrial extracts; v) 96-h exposure induced the over-expression of hsc/hsp70 proteins and, conversely, the down-regulation of cytochrome oxidase subunits II and IV. These observations, in addition to providing more information on the cellular and molecular aspects of the interaction between $CdCl_2$ and MDA-MB231 breast tumor cells, contribute to the comprehension of the intracellular molecular mechanisms implicated in the regulation of some mitochondrial proteins.

Histonic acetylation and methylation status in mouse embryos exposed to Meth and VPA: correlation with a possible VPA-related mechanism of teratogenesis-preliminary data.

ELENA MENEGOLA, MARIA LUISA BROCCIA, FRANCESCA DI RENZO, ERMINIO GIAVINI
elena.menegola@unimi.it

The teratogenic effects of valproic acid (VPA) on axial skeleton development have been recently correlated with histone hyperacetylation at the level of the axial embryonic organs (somites) at 1 h after treatment. Histone acetylation, as well as methylation, phosphorylation and ubiquitination are epigenetic processes involved in critical cell processes such as control of proliferation, differentiation and survival/cell death. Epigenetic regulation of the expression of genes controlling the axial pattern has been also demonstrated in embryos. Generally, histone acetylation is correlated to gene transcription, while methylation activates or inhibits transcription depending on the methylated residues. Literature data indicate the role of pre-treatment with methionine (Meth) as effective in reducing the frequency of neural tube defects related to VPA treatment, but worsening the effects of VPA in inducing embryolethality and developmental delays.
The aim of the present work is the study of the acetylation/methylation status in mouse embryos exposed *in utero* at E8 to L-Meth (70 mg/kg, intraperitoneal injection) or saline and after 30 min to VPA (400 mg/kg, intraperitoneal injection) or saline. Treated dams were sacrificed just before the VPA treatment (t0) or at 1 and 3 hours (t1, t3) after VPA treatment, embryos explanted, morphologically examined and processed for western blot or immunohistochemical analyses. Some females were sacrificed at term of gestation (E18), foetuses explanted, externally morphologically examined and processed for the double staining of bone and cartilage.
Axial abnormalities were confirmed after VPA treatment, no effects on skeleton were induced by Meth alone, while the double exposure to Meth and VPA caused a clear increase of the incidence of affected foetuses and a more severe pattern of the axial abnormalities in comparison to those induced by VPA alone. The pre-treatment with Meth seemed to be correlated to the maintenance of the hyperacetylated status at 1 and 3 h after treatment. Both VPA and Meth seemed to be correlated to the methylated status of H3K4 (Lys 4 of histone H3), involved in gene transcription activation. These molecular finding could be the mechanistic basis accounting for the effects of the double exposure to Meth and VPA on axial skeleton development.

Differential display analysis of gene expression in lizard embryos following cadmium exposure.

FRANCESCA TRINCHELLA, MARCELLO CANNETIELLO, PALMA SIMONIELLO, SILVANA FILOSA, ROSARIA SCUDIERO
sifilosa@unina.it
Dipartimento delle Scienze Biologiche, Università degli Studi di Napoli Federico II

In the natural environment, cadmium (Cd) is often found as a trace contaminant. Cd ions are highly reactive and, once in the cells, are able to modify various enzymatic processes (Bertin & Averbeck, Biochimie 88, 2006). Consequently, Cd is generally toxic for all living cells. Nevertheless, it is often difficult to determine clear relationships between analytical Cd m! easurements and its induced biological response. Actually, no ! data lang2057exist for wild terrestrial vertebrates. In an effort to address these questions, we proposed as a model study the developing lizard embryos. Lizard eggs present a thin and highly extensible parchment-like shell; they contain insufficient water to complete development and must absorb large amounts of water from the surrounding soil. Therefore, the potential exists for absorbing toxicants dissolved in soil pore water.
Screening for differentially expressed genes is a straightforward approach to study the molecular basis of cellular response to Cd-toxicity. The differential display-PCR (DD–

PCR) analysis is a powerful tool for analyzing the expression profiles of large numbers of genes.! angnp2057
In this study, DD-PCR technique was employed to identify genes whose expression was altered in lizard embryos following Cd-exposure. Fertilized eggs of the lizard *Podarcis sicula* were placed in Cd-contaminated soil (50mg Cd/Kg soil) and incubated at 25°C for 20 days.
PCR analysis using 76 primer combinations identified 18 differentially expressed gene fragments. Gene expression profiling showed 6 down-regulated and 12 up-regulated transcripts. Cloning and sequencing of the transcripts allowed us to identify 8 known genes. Characterization of these genes revealed that in lizard embryos Cd affected molecular pathways associate! d with fundamental processes for embryo development: membran! e trafficking, signal transduction, cytoskeletal organization, cell proliferation and differentiation. Cd also affected the expression of factors actively involved in the regulation of the transcription machinery.
Six fragments exhibited significant sequence similarity with genes encoding novel proteins or ESTs derived from other vertebrates. Finally, 4 cDNAs had no homology to known gene sequences, thus suggesting that may either encode not yet identified proteins, or correspond to untranslated regions of mRNA molecules.
Our findings confirm that Cd causes a severe perturbation of early embryo development in lizard and lead to an increased understanding of genes and biochemical pathways impaired by cadmium.

VPA-related axial skeletal defects and the induction of apoptosis in mouse embryonic tissues as possible pathogenic pathway

FRANCESCA DI RENZO, MARIA LUISA BROCCIA, ELENA MENEGOLA, ERMINIO GIAVINI
francesca.direnzo@unimi.it

The antiepileptic drug valproic acid (VPA) is a teratogenic agent both in humans and in animals. In rodents, VPA exposure during the embryogenesis induces neural tube defects and axial skeletal malformations. Recently, our research group showed a relationship between VPA-related axial malformations and histone hyperacetylation at the level of somites at 1 h after treatment. Histone hyperacetylation is one of the epigenetic controls of expression of genes involved in proliferation, cell cycle control and programmed cell death (apoptosis). VPA is an inhibitor of the histone deacetylases (HDAC), property related to its anti-neoplastic activity by inhibiting cell proliferation and activating apoptosis and differentiation.
The aim of this work is to investigate if VPA-induced teratogenic effects are related to histone hyperacetylation through the activation of apoptotic pathways in somites. CD-1 pregnant mice were intraperitoneally injected with VPA (400 mg/kg) or saline on day 8 of gestation. Embryos were explanted at 1, 3, 5, 9, 24 h after treatment and processed for western blot (WB) and immunohistochemical (IHC) analyses to detect proteins of interest or stained with the vital dye acridine orange to detect apoptotic cells. Some fixed embryos were stained to detect morphological abnormalities. Foetuses, explanted at term of gestation, were double stained for bone and cartilage to detect skeletal abnormalities. The proteins detected by WB and IHC were hyperacetylated histone H4, acetylated lysine, early apoptotic markers (p53 and casp3) and a HDAC active in adults and during the embryogenesis (SIRT-1).
A marked increase of the hyperacetylation level was detected at 1 h and was restricted to histones at the level of somites. A peak of p53 and casp3 was observed at 5 h mainly at the level of somites. At 9 h, acridine orange showed apoptosis at the level of somites. The expression of SIRT-1 was reduced till 5 h, and progressively restored at 9 and 24 h. Fused and irregular somites were observed at 24 h. At term of gestation, VPA induced axial abnormalities (fusions, duplications, homeotic respecification). In conclusion, the present data suggest a relationship between VPA exposure- effects on embryonic HDAC

expression- histone hyperacetylation- activation of apoptosis- somite abnormalities- axial malformations. The proposed VPA-related mechanism and pathogenic pathway in embryos are respectively the HDAC inhibition and the activation of the apoptotic pathway.

Biomolecular characterization of human glioblastoma cells in primary culture differentiating and anti-angiogenic effects PPARγ ligands

ELISABETTA BENEDETTI .*, RENATO GALZIO °, BARBARA D'ANGELO *, BENEDETTA CINQUE ^, ALESSANDRO RICCI §, GIULIO LAURENTI *, DANIELA LOMBARDI ^, MARIA GRAZIA CIFONE ^ AND CIMINI ANNAMARIA* ebenedetti3@virgilio.it
* DIP.. BIOLOGIA DI BASE E APPLICATA, L'AQUILA
° DIP. SCIENZE CHIRURGICHE, L'AQUILA
^ DIP. MEDICINA SPERIMENTALE, L'AQUILA
§ OSPEDALE S. SALVATORE, L'AQUILA

Gliomas are the most commonly diagnosed malignant brain primary tumors. Prognosis of patients with high grade gliomas is poor and poorly improved by radiotherapy and chemotherapy. Several studies have reported the antiproliferative and/or differentiating activities of some lipophylic molecules on glioblastoma cells. Some of these activities in cell signalling are mediated by a class of transcriptional factors referred to as peroxisome proliferator-activated receptors (PPARs). PPARγ has been identified in transformed neural cells of human origin and it has been demonstrated that PPARγ agonists decrease cell proliferation, stimulate apoptosis and induce morphological changes and expression of markers typical of a more differentiate phenotype in glioblastoma and astrocytoma cell lines. These findings arise from studies mainly performed on long term cultured transformed cell lines. Such experimental models do not exactly reproduce the *in vivo* environment since long-term culture often results in the accumulation of further molecular alterations in the cells. To be as close as possible to the in vivo conditions, in the present work we investigated the effects of PPARγnatural (conjugated linoleic acid, CLA) and synthetic ligands on the biomolecular features of primary cultures of human glioblastoma cells derived from surgical specimens. Treatments with natural or synthetic ligands of PPARγ decrease the expression of undifferentiation markers such as CD133, nestin, fibronectin, while increasing the expression of differentiation markers such as A2B5, GFAP, ß-catenin, N-cadherin. Moreover, the treatments significant reduce the angiogenic potential of glioblastoma cells. In fact, both CLA and PPARγ agonist treatments led to a significant decrease of the VEGF isoforms, thus indicating that PPARγ is able to inhibit the angiogenetic pathways in glioblastoma. In conclusion, in this work we focused on the anti-neoplastic effects of PPARγ agonists in an *in vitro* model system more strictly resembling the human glioblastoma natural environment than cell lines. Our data clearly indicate that PPARγ natural and synthetic ligands promote a combination of growth inhibitory, pro-apoptotic, antiangiogenetic and cell differentiating effects on glioblastoma cells, suggesting that PPARγ agonists might be considered as novel therapeutic drugs for patients affected by malignant gliomas. Moreover, CLA, a natural occuring fatty acid, which is known to cross the blood brain barrier, appears suitable as chemopreventive agent as it does not need clinical trials since it is already used on humans. On the other hand, the γ agonist, once experimented and validated on man, may represent a useful coadjuvant in glioblastoma therapy and in the prevention of recurrencies.

Autophagy and programmed cell death in the IPLB-LdFB insect cell line: new clues from proteomics

DAVIDE MALAGOLI[1], FEDERICA BORALDI[2], GIULIA ANNOVI[2], DANIELA QUAGLINO[2], ENZO OTTAVIANI[1]
davide.malagoli@unimore.it
[1] Department of Animal Biology, University of Modena and Reggio Emilia, Via Campi 213/D, 41100, Modena, Italy
[2] Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Via Campi 287, 41100, Modena, Italy.

Autophagy is an ancient process based on the activity of a pool of genes conserved from yeasts to metazoans. The physiological role of autophagy in providing energy during cell starvation is commonly accepted, while debate continues about the occurrence of autophagy as a specific means that is activated to achieve cell death. The IPLB-LdFB insect cell line, derived from the larval fat body of the lepidopteron *Lymantria dispar* (gipsy moth), represents a suitable model to address this question, as both autophagy and apoptotic cell death can be induced with different stimuli. Morphological observations indicated that the medium conditioned by IPLB-LdFB cells treated with the ATPase inhibitor oligomycin A triggers cell death in untreated cells. Ultrastructural experiments demonstrated that the triggered cell demise is achieved mainly through autophagy. ATP dosage excluded that the pro-autophagic effects of conditioned medium is related to residual oligomycin A. Two-dimension electrophoresis identified several proteins whose presence in the conditioned medium was altered after the exposure to oligomycin A. Subsequent analysis with mass spectrometry led to the identification of 11 peptides displaying a possible role in the onset of autophagic cell death that follows the treatment with oligomycin A. Among them, an imaginal disk growth factor-like protein is a plausible candidate to explain the pro-autophagic effects of oligomycin A. On the whole, our data give new insights in the molecular promoters of autophagy and support the view of autophagic cell death as a proper programmed cell death in insects.

Type V collagen regulates apoptosis-related gene expression in breast cancer cells

ROSALIA SIRCHIA, ALESSANDRA LONGO, CLAUDIO LUPARELLO
clupar@tin.it
Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo.

Type V collagen is a "minor" stromal component accounting for <1% of total collagens in the adult tissues, whose amount increases up to 10% in cases of ductal infiltrating carcinoma (DIC) of the breast [1]. When the DIC cell line 8701-BC was cultured onto type V collagen, this substrate, i) allowed adhesion of only a part of the cell population whose growth and motility was drastically reduced, ii) modified cell morphology, iii) induced DNA fragmentation, iv) affected the expression levels of apoptosis- and stress response genes, up-regulating *BclxS*, *Bad*, *Dap kinase*, *hsf1*, *hsp75*, *caspase-1*, *-5*, *-8*, *-9*, *-14* whilst down-regulating *Bcl2*, *Bclxß* and *hsp60*, and, v) up-regulated caspase activation [2-5], thereby being endowed of potential anti-tumoral properties deserving additional studies. We searched for other genes selectively expressed after cell adhesion to type V collagen by "differental display"- and semiquantitative PCR assays and observed the collagen-dependent up-regulation of *CAPN2*, coding for the large subunit of m-calpain, a cell death-involved cystein protease, and the down-regulation of *protein kinase C*, *isoform η* (*PKCη*. Noteworthy, it is known that *PKCη* expression level decreases during the physiologic involution of rat mammary gland [6], whilst its up-regulation actively inhibits the onset of apoptosis in different cell models. To ascertain whether *PKCη* down-regulation might be involved in collagen-induced cellular modifications, we tested the effect of transfection with antisense *PKCη* oligoon cells seeded onto "physiologic" type IV collagen substrates. Our data demonstrate that

transfected cells show similar changes in cell phenotype and gene expression, even in the absence of type V collagen, thus suggesting the involvement of PKCη in apoptosis-related events also in neoplastic cells, and further support the importance that alterations of stromal composition may have on cell reprogramming of gene expression.
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Signal transduction pathways involved in PPARß-induced neuronal differentiation

BARBARA D'ANGELO*, ELISABETTA BENEDETTI *, SILVIA DI LORETO °, LOREDANA CRISTIANO *, MARIA PAOLA CERÙ * AND ANNAMARIA CIMINI *
cimini@univaq.it
*Dip. Biologia di base e applicata Università dell'Aquila
° ITOI, CNR, L'Aquila

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. PPARs exhibit a broad but isotype-specific tissue expression pattern which can account for the variety of cellular functions they regulate. PPARβ/δ is expressed more ubiquitously and earlier during fetal development. PPARs are activated by a wide range of naturally occurring or metabolized lipids derived from the diet or from intracellular signaling pathways, which include satured and unsatured fatty acids and fatty acid derivatives such as prostaglandins and leukotriens. We have previously shown in SH-SY5Y neuroblastoma cells that oleic acid (OA) and of a specific synthetic PPARß agonist (GW) reduce cell growth, increase the expression of neuronal differentiation markers and reduce parameters responsible for the malignancy such as adhesion, migration, invasiveness and TrkB expression, thus suggesting a role for PPARß in neuronal differentiation. In the present work, we studied the signal transduction pathways involved in neuronal differentiation such as the MAPK ERK1,2 and BDNF/TrkB. The results obtained demonstrate that both OA and GW effects on cell growth and differentiation are mediated by PPARß/δ since they were reduced or even completely reverted by PPARß siRNA. Moreover, PPARβ/δ-induced neuronal differentiation depends on activation of the ERK1/2 trasduction pathway. In fact, similarly to PPARß/δ gene silencing, the inhibition of ERK1/2 pathway blocks the neurite outgrowth and the differentiation program induced by OA and GW. Besides confirming both ERK1/2 and PPARß/δ role in neuronal differentiation, our data demostrate that, in neuroblastoma, the neuronal differentiation program triggered by PPARß/δ activation implies subsequent ERK1/2 activation Recent reports demonstrate that high levels of expression of BDNF and TrkB in its full length form also play important roles in the biology and clinical behaviour of neuroblastomas, as they are highly expressed in less favorable, more aggressive neuroblastomas In SH-SY5Y neuroblastoma cell line both OA and GW decrease full-lenght TrkB mRNA thus indicating that they may exert their antiproliferative and differentiating activity by downregulating this pathway. Besides their use as differentiating therapy, the ability of OA and GW to downregulate TrkB expression suggests to use these compounds as therapeutich tools to halt the TrkB- dependent drug-resistance of neuroblastomas.

Cloning and developmental expression analysis of synapsin genes in two basal chordates: *Branchiostoma lanceolatum* and *Ciona intestinalis*

SIMONA CANDIANI, LUCA MORONTI, ROBERTA PENNATI, FIORENZA DE BERNARDI, FABIO BENFENATI, MARIO PESTARINO
roberta.pennati@unimi.it

Synapsins are a family of neuron-specific phosphoproteins that play a key role in neurotransmitter release. Moreover, these molecules participate during development in the regulation of neurite outgrowth, axon elongation and synaptogenesis (Ferreira and Rapoport, 2002). Synapsins have been identified in representative organisms from major phyla throughout the animal kingdom (Kao et al.1999). In invertebrates only one synapsin gene appears to exist. In mammals, three synapsin genes have been identified, and each one gives rise to different alternatively spliced isoforms. To better understand the evolution of synapsin gene family, we cloned and sequenced synapsin genes from two invertebrate chordates, the amphioxus *Branchiostoma lanceolatum* (Cephalochordata) and the ascidian *Ciona intestinalis* (Tunicata), and compared them to known synapsin sequences from mammals and other invertebrates. Both the genomes contain a single synapsin gene that shows a high degree of conservation in exon/introns organization to those found in mammals. Moreover, as in mammals, in these basal chordates some introns of synapsin gene locus encode tissue inhibitor of metalloproteinases (TIMP). The amphioxus and the ascidian synapsin proteins contain all types of conserved domains (A, C, and E) characteristic of synapsins. By whole mount in situ hybridization we studied the temporal and spatial expression pattern of synapsin gene during development. In *C. intestinalis* larva, the synapsin gene is expressed in most neurons of the central and peripheral nervous system. In the embryo synapsin transcripts are detected in the floor of the posterior sensory vesicle, where the main groups of neurons are differentiating, in the visceral ganglion and in the nerve fibers of the posterior tail, possibly marking the areas of active synaptogenesis. Both in *C. intestinalis* and in amphioxus, synapsin genes are first expressed in regions were cholinergic neurons are differentiating. The analysis of the expression of these genes will help to elucidate the timing of neural differentiation in these basal chordates.

Hsp70 functions: inside and outside the cell

G. TURTURICI, F. GERACI , M.E.CANDELA , G. GIUDICE AND G. SCONZO gasc@unipa.it
Dipartimento di Biologia Cellulare e dello Sviluppo, Universita' di Palermo

HSP70 expression is induced in response to several kinds of cellular stress in all living organisms. We demonstrated that A6 mesoangioblast stem cells have a constitutive HSP70 expression which may protect them from sudden cellular stresses, but no significant HSF-binding activity was found, suggesting that constitutive HSP70 expression is HSF independent. hsp70.1 and hsp70.3 are the major stress-inducible genes. Mesoangioblaststs are vessel-derived stem/progenitor cells which can differentiate into different cell types of solid mesoderm such as muscle and bone.
In order to understand the role of HSP70 constitutive expression in mesoangioblasts, we have carried out stable RNA interference of hsp70.1/hsp70.3. A6 cells were transfected with phsp70.1/3 silencing vector. Among all the clones we chose one (A6-NM3) with a 30% lower level of hsp70.1 and a 24% lower level of hsp70.3 and with a 55% Hsp70 knockdown. We investigated the effect of hsp70.1/3 silencing on cell growth in A6-NM3 and A6 cells. We measured the duplication time and found that A6 cells have a duplication time of 16 hours as previously described for mouse mesoangioblasts, while A6-NM3 cells have a duplication time of 23.4 hours. This datum confirms the proliferation assay and indicates a possible role for the basally synthesized HSP70.
We explored whether A6 stem cells have the ability to release proteins out of the cells by

vesicles shedding. To ascertain this shedding, A6 cells have been cultured in three-dimensional collagen gels. Staining with fluorescein conjugated-phalloidin showed vesicular structures near the cells. Inside the vesicles we found actin, filamin and tubulin, but not lamp1. We also demonstrated that they contain both Hsp70 and Hsc70, but Hsp70 is preferentially released. Double fluorescence assays showed a co-localization between Hsp70 and actin both near cell membrane, and on A6 cell microfilaments. Immunoprecipitation assay confirmed the immunofluorescence data. Actin involvement on Hsp70 release has been verified. A6 cells have been treated with cytochalasine or nocodazole. Hsp70 amount released by cytochalasine treated cells was decreased of 32%, although vesicle shedding was unaffected by treatment. Different results were obtained from nocodazole treated cells. Percentage of Hsp70 content into shed vesicles was unchanged respect with control vesicles. Hsp70 constitutive expression in A6 stem cells may have functions inside the cells and in external space.

Endocannabinoids and stress in teleost fish

ALBERTA MARIA POLZONETTI-MAGNI[1,2], FRANCESCO PALERMO[1,2], ERICA COTTONE[3], MATTEO VIRGILI[1], GILBERTO MOSCONI[1], MARIA FOSCA FRANZONI[3]
alberta.polzonetti@unicam.it
[1] Dipartimento di Scienze Morfologiche e Biochimiche Comparate, Universita' degli Studi di Camerino, via Gentile III da Varano, 62032 Camerino (MC), Italia.
[2]Centro Universitario di Ricerca per lo Sviluppo e la Gestione delle Risorse dell'Ambiente Marino e Costiero (UNICRAM), Università degli Studi di Camerino, 63039 San Benedetto del Tronto (AP), Italy.
[3] Dipartimento di Biologia Animale e dell'Uomo, Università degli Studi di Torino, 10123 Torino, Italia

Endogenous cannabinoids, through the cannabinoid receptor CB1, are involved in the modulation of adaptive responses to environmental conditions, and appear to be pivotal in some neuroendocrine mechanisms such as the regulation of vertebrate reproduction, stress, and food intake.
In the context of environmental endocrinology, the endocrine disruptors merit considerable attention, since they induce deep changes in the endocrine system; in the case of xenoestrogens, they interfere not only with the hypothalamus-pituitary-gonadal axis, but also with the neuroendocrine system regulating responses to environmental stress.
Sexually immature sole (*Solea solea*) were exposed to different concentrations of a xenoestrogenic compound, 4-nonylphenol (4-NP) or estradiol-17β (E2). After 3 and 7 days of treatment, immunological techniques were used to measure the changes of plasma vitellogenin and sex steroids, as indicators of estrogenic effects induced by 4-NP, while plasma cortisol was measured as an indicator of stress. Moreover, using semiquantitative RT-PCR, it was found that both E2 and 4-NP modulated the expression of CB1 receptor subtypes in the brain.
In addition, in investigations into the possible influence of fasting and re-feeding on the expression of CB1 mRNA levels in the goldfish brain, it was found that food deprivation induced an increase of CB1 expression levels, and also that intraperitoneal anandamide injections affect CB1 mRNA as well as NPY.
Our results support the involvement of CB1 receptors in the modulation of different types of stress responses, and suggest that CB1 receptor subtype expression may be serve as a biomarker for xenoestrogenic effects.

Cloning and Characterization of T-cell ReceptoR Gamma gene in sea bass, *Dicentrarchus labrax*

RITA PIERGENTILI, ANNA GRAZIA FICCA, ELISABETTA CACCIA, GIUSEPPINA CECCARELLI, MARIA AZZURRA MISSINATO, LUCIA MASTROLIA & NICLA ROMANO
nromano@unitus.it
Department of Environmental Sciences, Tuscia University, Viterbo, Italy

T cell receptor (TcR) is responsible for the recognition of self and foreign cells in jawed Vertebrates. In mammals, TcRs are heterodimeric, consisting of either α/β or γ/δ polypeptide combinations. Differently to the α/β T cells, γ/δ T cells appear to recognize Ags in a manner similar to the direct Ag recognition processes of immunoglobulin, and therefore do not require specialized Ag presentation by MHC. The structure and expression of genes encoding molecules homologous to mammalian α/β TcR have been identified from almost all vertebrate classes, including birds, amphibians, teleosts and elasmobranches. Recently, γ/δ TcR homologues have been also identified in several teleosts [Nam et al. 2003, J Immunol.170:3081-3090; Shang et al.,2008, Fish Shellfish Immunol. 24:412-425].

In this study, for the first time, the cloning and characterization of a partial cDNA encoding for TcRγ chain in the sea bass *Dicentrarchus labrax* obtained by RT-PCR strategy was reported. The length of the partial TcR γ cDNA is 851 bp and encodes 283 amino acids, including part of the Vγ region (67 amino acid residues), the core motif FGXG in J γ segment and the 191 aa long C γ region containing the characteristic CX6PX6WX45C motif with to conserved cysteine residues that form an intra-chain disulphide bond. Sequence analysis showed that sea bass TcR γ chain exhibits highest identity with *Paralichthys olivaceus* (V γ region 53%, J γ region 80%, C γ region 49%) and *Takifugu rubripes* (V γ region 54%, J γ region 76%, C γ region 41%). RT-PCR analysis demonstrated that the expression of TcR γ gene was detectable in examined lymphoid tissues (thymus, head kidney, spleen, gut and gills) of 8-12 month sea bass.

Developmental expression of antimicrobial peptides in zebrafish (*Danio rerio*)

ELISABETTA CACCIA, GERARDO R. VASTA*, ANNA GRAZIA FICCA, PATRICIA STRICKLER-DINGLASAN* AND NICLA ROMANO
e.caccia@unitus.it
*Department of Environmental Sciences, Tuscia University, Viterbo, Italy; *Center of Marine Biotechnology, UMBI, University of Maryland, Baltimore, Maryland (USA).*

Antimicrobial peptides (AMPs) are wide distributed among metazoans and their expression can be constitutive or inducible. AMPs either kill or inhibit the proliferation of microorganisms, including bacteria, fungi, and eukaryotic parasites. Although extensively studied, little is known about their temporal or spatial expression. The aim of this study was to identify and characterize novel components of the zebrafish AMP repertoire during development and in adult. Taking advantage of the expressed sequence tag (EST) databases, we cloned and characterized seven cDNAs encoding for members of antimicrobial peptides expressed in various tissues: (1) mucosal (parasin I and its specific protease Chatepsin D); (2) tissue-expressed antimicrobial peptide (Defensin beta-DB1, LEAP 2); 3) circulating phagocytes (bactericidal permeability-increasing protein, BPI); (4) the neuroendocrine/ immune system–related peptides, chromogranin A and B. Specific primer sets enabled the RT-PCR amplification of partial or the full-length AMP sequences from total RNA extracted from 24 hrs, 5 days and adult zebrafish. Amplicons [ranging from 242 bp (*dbi*) to 504 bp (*cgb*)] were cloned and sequenced, and the identity of the zebrafish antimicrobial peptides was confirmed by alignment of the deduced amino acid sequences of AMPs from other fish or mammalian species. Temporal expression of the AMPs was analysed by semi-quantitative RT-PCR in oocytes, embryos, and adult zebrafish. Both CgA and CgB transcripts were evident from 48 hours post fertilization (pf). Cathepsin D transcripts were detectable from 0 hr pf onwards, with a more intense band evidenced at day 12. Both Leap-2 and

DB1 remained undetectable until the adult stage. The mature mRNA of *zf* BPI was detectable at 12 days pf. AMPs may represent useful alternatives to antibiotics for therapeutic intervention in aquaculture.

Transcriptional analysis of tyrosinase gene expression during *Bufo bufo* development

OSVALDO ZARIVI, ANTONELLA BONFIGLI, SABRINA COLAFARINA, PATRIZIA CESARE, MICHELE MIRANDA
michele.miranda@aquila.infn.it
Department of Basic and Applied Biology, University of L'Aquila, via Vetoio, L'Aquila Italy

To follow the transcriptional expression of tyrosinase gene during the development of *Bufo bufo* and to verify if transcription and translation are contemporary we have first cloned and sequenced the tyrosinase cDNA, and successively prepared some primers to test by RT-PCR the levels of tyrosinase mRNA during development. Previously we had measured spectrophotometrically the tyrosinase activity during *Bufo bufo* development by extracting the enzyme from melanosomes and characterizing it by electrophoresis with a long and expensive work. The preparation of a polynucleotidic labeled probe for tyrosinase mRNA will allow to test the transcriptional expression of the gene. Moreover immunohistochemical studies will allow to localize the protein and the intracellular route from ribosomes to Golgi's apparatus and then to melanosomes.

Internalization of TSH receptor and Thyroblogulin in thyroid cells

SIMONA PICCHIETTI, CRISTINA BELARDINELLI, ANNA RITA TADDEI , ANNA MARIA FAUSTO , MARIO ROSSI AND FRANCO GIORGI
giorgif@biomed.unipi.it

G protein-coupled receptors (GPCRs) control a plethora of metabolic responses by interacting specifically with hormones, neurotransmitters and growth factors. Upon interaction, these receptors uncouple from their associated G protein and activate a cascade of enzymes downstream. The TSH receptor (TSHr) plays a key role in the metabolic activity of the thyroid gland by controlling the cAMP intracellular concentration. We demonstrate that the insecticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) acts as thyroid disruptor by diminishing TSH receptor activity. Here we show that exposure to TSH leads to an intracellular accumulation of cAMP and to the internalization of the receptor itself. The TSH receptor was probed by inserting a short tetracysteine sequence along its C-terminal domain to make it specifically fluorescent through interaction with the membrane-permeant fluorescein derivative (FlAsh). TSH receptors were also localized immuno-cytochemically with specific anti-TSHr antibodies in CHO and COS-7 cells exposed for various lengths of time to TSH. By this procedure we demonstrate that exposure to DDT inhibits the endocytic internalization by retaining the TSH receptor along the plasma membrane. As a control, the effects of DDT were verified on CHO cells transfected with the A2a receptor, whose internalization was not inhibited by DDT exposure. We additionally explored whether DDT and TSH could also interfere with other cell parameters by evaluating the membrane conductance under various exposure conditions.
Finally, various cell types, including FRTL-5, IPTC and CHO cells, were exposed to gold-tagged thyroglobulin to verify whether endocytosis in these cells could depend on specific receptors and is eventually mediated by TSH exposure. Interestingly, only FRTL-5 cells proved capable of undergoing transcytosis, by internalizing along the apical pole and releasing it along the basal pole. Other cell types, although still capable of internalizing gold-thyroglobulin, retain it intracellularly or release it to a much less extent. Results are interpreted as indicating thyroglobulin internalization as a rather complex phenomenon. Even though endocytosis may occur in bulk, internalization is likely to require specific intracellular processing along the lysosomal pathway.

Differentiation of interrenal cells in the developing adrenal gland of *Testudo hermanni*

FIORENZA ACCORDI, CLAUDIO CHIMENTI, VALENTINA PATRIZIA GALLO, ROCCO LIGUORI AND HARRY MANELLI
Dipartimento di Biologia Animale e dell'Uomo, Università "La Sapienza" di Roma – Viale dell'Università, 32 – 00185 Roma
fiorenza.accordi@uniroma1.it

The morphology, histology, histochemistry and immunohistochemistry of the adrenal gland were examined during development of *Testudo hermanni*. The main purpose was to investigate the ontogenetic origin of the interrenal steroidogenic cells and their relationships with the chromaffin cells and the renal system; this latter plays an important role during development, as it shares the same mesodermic origin with the interrenal cells. In the course of vertebrate evolution the adrenal gland organizes in contact with the renal parenchyma and shows a strong evolutionary trend towards a concentrated arrangement starting from conditions of great dispersion. The peritoneal mesoderm gives rise to steroidogenic tissue at a more caudal level with respect to the first kidney that develops in all vertebrates (the pronephros), while the chromaffin cells, the other constituent of the adrenal gland, reach this area, migrating from the neural crests. In some vertebrates, like chelonian reptiles, the adrenal gland always retains the original position external to the mesonephric or the metanephric kidney.
Few data are available on the origin and differentiation of interrenal cells in chelonians; this topic was therefore investigated in the present work.
The presence of steroidogenic cells was tested by immunoreaction with 3ß-hydroxysteroid-dehydrogenase antibody; the first evidence occurred at the end of the early embryonic period at stage 17-18, in a small group of cells on the intermesonephric wall. The reaction was scarce and localized near the medial edge of the mesonephric kidney. In the following stages, until the hatching, the presence of immunoreaction was scarce, although the adrenal gland seemed well developed, composed of interrenal cords surrounded by small groups of chromaffin cells. The weak reaction for 3ß-hydroxysteroid-dehydrogenase could be due to the little amount of cytoplasm, as these cells are very rich in lipid droplets.
Electron microscopic observations confirmed the immunohistochemical data: in the early embryonic period of development, stage 17-18, few steroidogenic cells were observed: they had irregular shape, cytoplasmic projections extending into the intercellular space and high nucleo-plasmatic ratio. In the cytoplasm few lipid droplets occurred. In the following stages steroidogenic cells were arranged in larger groups. Their shape became more regular, the cytoplasm was very rich in lipid droplets and in vesicular mitochondria. Different aspects in the morphology of nucleus, SER and RER, lipid droplets and mitochondria were observed; their variation probably reflected changing secretory activity. The presence of cilia in the interrenal cells was occasionally observed: these may account for the origin from celomic epithelium.

Transcriptional control of human steroid sulfatase

ALESSIA NARDI, ELENA POMARI , LORENZO COLOMBO , LUISA DALLA VALLE
dallavalle@unipd.it
Dipartimento di Biologia, Università di Padova, via U. Bassi 58/b, 35121 Padova, Italy.

During pregnancy, steroid sulfatase (STS) in the syncytiotrophoblast is a key enzyme freeing sulfated androgens from fetal adrenals for conversion into placental estrogens. Postnatally, this enzyme is required for the intracrine conversion of adrenocortical dehydroepiandrosterone sulfate into biologically active androgens and estrogens, presumably involved in tissue maintenance and regeneration. The entire human *STS* gene spans over approximately 200 kbp of which the first 100 kbp include the regulatory region, while the STS-coding region is

located downstream. Previous studies indicated that *STS* in the placenta is regulated by a first exon (exon 1a) with alternative transcription start sites (TSS). As an advancement, we have analyzed the *STS* expression in 16 human tissues and cell lines, by means of 5'-RACE analysis. Seven different promoters were found to drive *STS* expression, giving rise to transcripts with unique 5'-untranslated termini. While placental exon 1a and exon 0d, found in a prostatic cell line, are partially coding, the other 5 first exons are all untranslated. Three of these (exons 0a, 0b and 0c) are spliced to a common partially coding exon 1b, the exon 0d is spliced to coding exon 1e, whereas the other two (1c and 1d) are spliced to exon 2, that occurs in all transcripts. Since these transcripts present different putative translation initiation codons, in frame with the following coding region of *STS*, the encoded proteins may contain N-terminal regions of different length, but all located within the signal peptide, which is cleaved post-translationally to obtain the mature STS protein. Sequence analysis of the 5'-flanking region of each first exon revealed a lack of TATA box or initiator element, thus accounting for the use of multiple TSS in nearly all first exons. The proximal promoters were found devoid of consensus elements for transcription factors related to steroidogenesis. The finding of alternative first exons and multiple TSS shows that the transcriptional regulation of *STS* is more complex than previously thought and suggests that different promoters and trans-acting factors are probably involved in the tissue-specific expression of this gene.

Cellular trafficking of BDNF and neuronal cytoarchitectonics

VICARIO A[1], BAJ G[1], LEONE E[1], CHAO M[2] AND TONGIORGI E[1]

[1]BRAIN Centre for Neuroscience. Dipartmento di Biologia. Università di Trieste. 34127 Trieste. Italy. [2]New York University – Skirball Institute, NY-USA.

The neurotrophin Brain Derived Neurotrophic Factor (BDNF) is a potent morphoregulatory agent that is necessary for the maintenance of the structure of neuronal dendritic arborisation. BDNF is encoded by multiple transcripts, generated by alternative splicing of the 5' non translated region (5'UTR), whose function is unknown. We have recently found that in rat hippocampal neurons, some BDNF transcripts are segregated in the cell soma while others extends into the dendrites by transport mechanisms involving RNA binding proteins that are extremely well conserved from Drosophila to man. Accordingly, we have analyzed the cellular trafficking of BDNF mRNA in its dynamic, molecular and functional aspects.

From the dynamic point of view, BDNF mRNA is transported in dendrites at an instantaneous speed ranging between minim of 260um/h and maximal of 936um/h. It is possible to observe both slow and fast anterograde and retrograde movements as well as stationary movements in which RNA granules oscillate around a fixed point for short range distances.
From a molecular point of view, we identified in the rat BDNF mRNA, putative binding sequences for 10 RNA binding proteins and we show that they are involved in BDNF mRNA transport.
Finally, from a functional point of view, we found that translation of BDNF mRNA in different regions of the dendrites has a local effect on cytoarchitectonic of both developing and mature neurons. In particular, in young neurons, the somatic isoforms determinate the number of dendrite branchings and spines in proximal dendritic segments but have no effect on distal ones. Conversely, the dendritic isoforms exert their morphogenic

effect only on distal dendritic compartments. The dendritic tree of mature neurons resulted to be much more stable and dendritic BDNF splice variants with exons 2 and 4 showed a significant effect restricted only to secondary and tertiary dendrite branchings.

sFRP-3 binds EGF in the extra-cellular space: effects on proliferation, differentiation and embryonic morphogenesis.

CESARE GARGIOLI[1], STEFANO.M.CANNATA[2], SERGIO BERNARDINI[2], S. BRUNELLI[3] AND GIULIO COSSU [4]

[1]Institute of Cell Biology and Tissue Engineering. San Raffaele Biomedical Science Park of Rome; [2]Dept. of Biology, University of Tor Vergata; [3]Dept. of Experimental Medicine, University of Milan-Bicocca; [4]Dept. of Biology, University Milan.
cesare.gargioli@spr-r.it, cannata@uniroma2.it, cossu.giulio@unimi.it

sFRP-3 (soluble Frizzled Related Protein 3), a soluble antagonist of Wnts blocks Wnt-8 induced axis duplication and induces axis truncation in *Xenopus* embryos. sFRP-3 also inhibits myogenesis in amphibians and mammals. Here we report that sFRP-3 blocks fibroblast proliferation and foci formation induced by EGF but not other growth factors. It also reverts EGF-mediated inhibition of hair follicle development in the ectoderm. Conversely, EGF reverts the inhibition of somitic myogenesis and axis truncation in *Xenopus* embryos caused by sFRP-3. In vitro experiments demonstrated a direct binding of EGF to sFRP-3 both on heparin and on the surface of CHO cells where the molecule had been membrane anchored.
Thus, sFRP-3 and EGF reciprocally inhibit their effect on cell proliferation, differentiation and morphogenesis and indeed are expressed in contiguous domains of the embryo, suggesting that in addition to their canonical ligands (Wnt and EGF receptor, respectively) these molecule bind to each other and regulate their activities during embryogenesis.

Indice degli Autori