



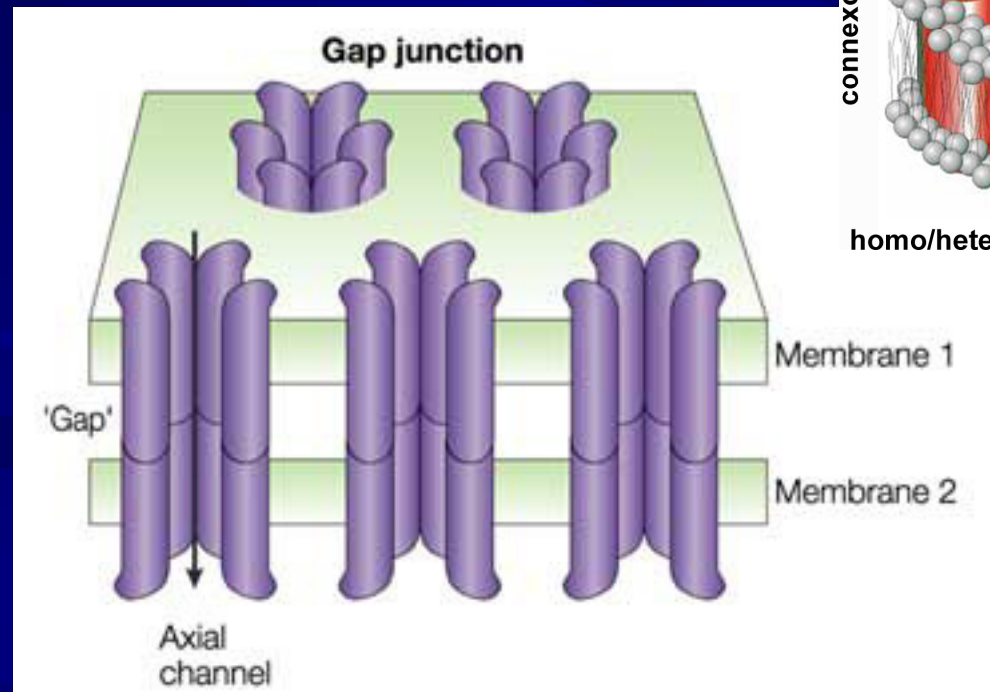
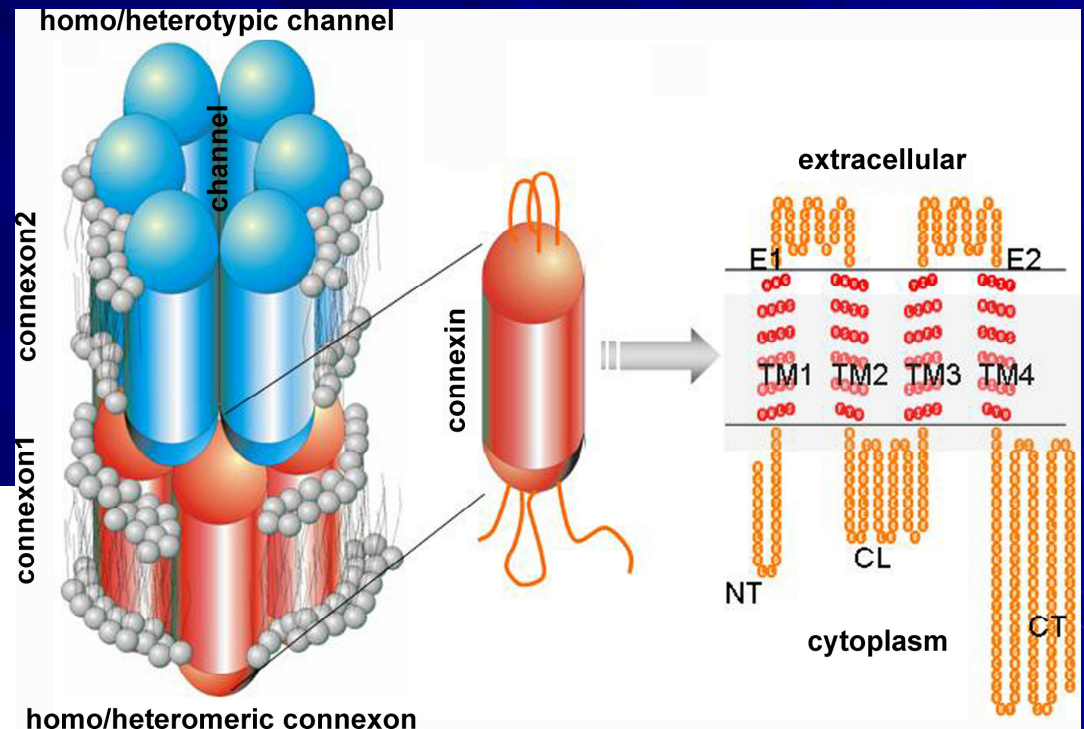
Rolul conexinelor in integrarea transcriptomica a unor procese vitale

Dumitru Andrei Iacobas
Dominick P Purpura Department of Neuroscience
Albert Einstein College of Medicine
New York, USA
diacobas@aecom.yu.edu

Jonctiune distanta – Canal intercelular - Connexon – conexina

O familie de ~20 de proteine care asigura la vertebrate **continuitatea citoplasmatica** intre celule prin formarea de canale intercelulare de jonctiune distanta (gap junction channels).

Conexinele sunt topologic asemanatoare cu inxinele de la organismele nevertebrate si panexinele.



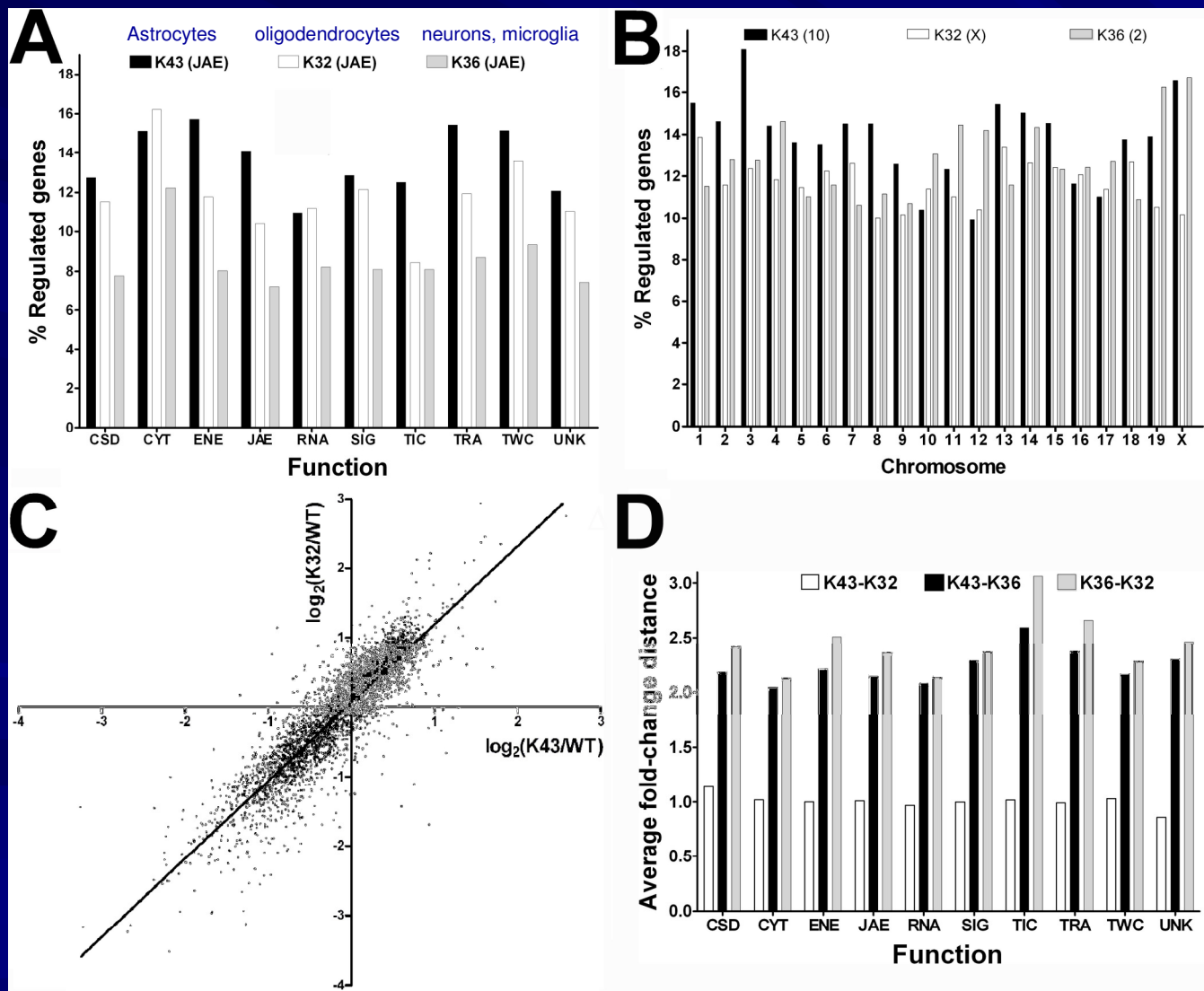
Canalele intercelulare pot asigura **continuitatea transcriptomica** intre celulele cuplate prin difuzia de:

- Ioni si molecule de semnalizare (Ca^{2+} , IP_3 , cAMP) care pot modula exprimarea genelor prin modificarea fosforilarii unor factori de transcriere (Stains & Civitelli, 2005; Stains et al. 2003)

- Antigeni cu masa moleculara < 1.8kD (Neijssen, 2005)

- ARN de interferenta (Valiunas et al., 2005).

Blocarea exprimării unei conexine alterează exprimarea a sute de gene



Genele afectate sunt localizate în toți cromozomii și codifică proteine din toate categoriile funcționale:

- CSD** = Cell cycle, shape, differentiation, death,
- CYT** = cytoskeleton,
- ENE** = Energy metabolism,
- JAE** = Cell junction, Adhesion, Extracellular matrix,
- RNA** = RNA Processing,
- SIG** = Cell Signaling,
- TIC** = transport of small molecules and ions into the cells,
- TRA** = Transcription,
- TWC** = transport of ions/molecules within the cells,

UNK = function not yet assigned.

Similarities and differences between the transcriptomic alterations in *Gja1*^{-/-}, *Gjb1*^{-/-} and *Gja9*^{-/-} brains compared to wildtype brain

Pan-glial transcriptomic network?

Iacobas et al., PBM 2007

Analiza de coordinatie

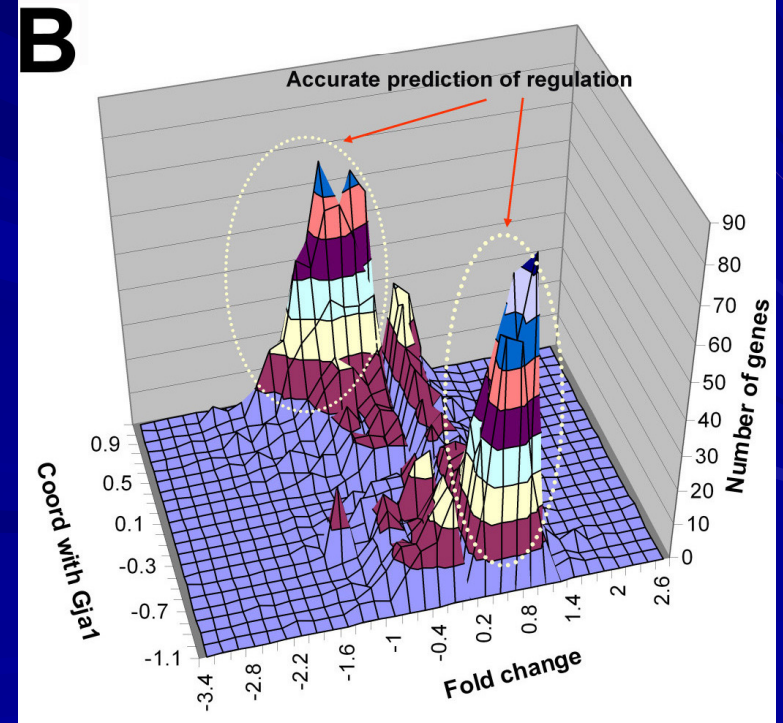
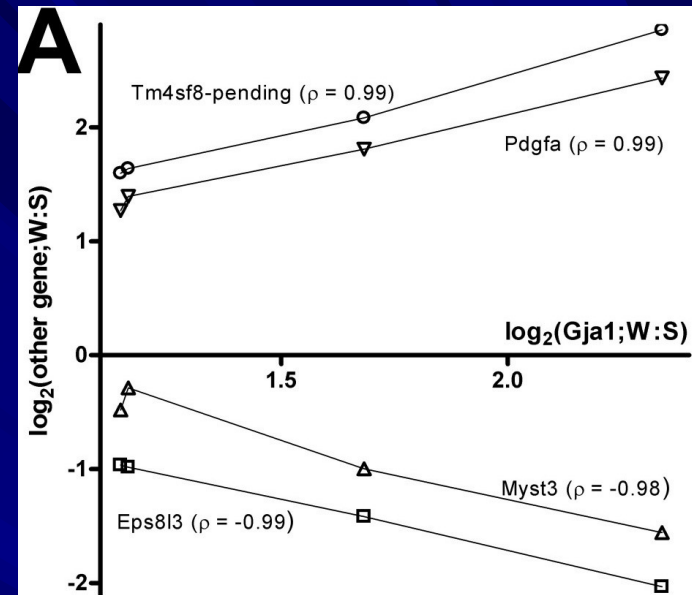
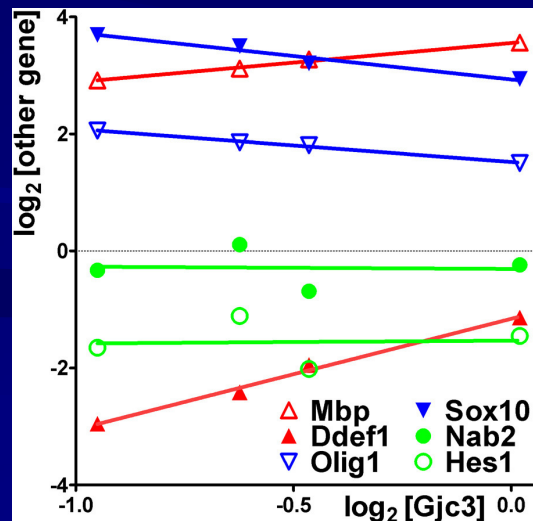
A. Examples of genes synergistically and antagonistically expressed with Cx43 (Gja1) in wildtype brain.

Pdgfa = platelet derived growth factor, alpha and Tm4sf8-pending = transmembrane 4 superfamily member 8, Myst3 = MYST histone acetyltransferase (monocytic leukemia) and Eps8l3 = ESP8-like 3.

B. Prediction of regulation in the Gja1 null brain from the coordination degree with Gja1 in the wildtype brain.

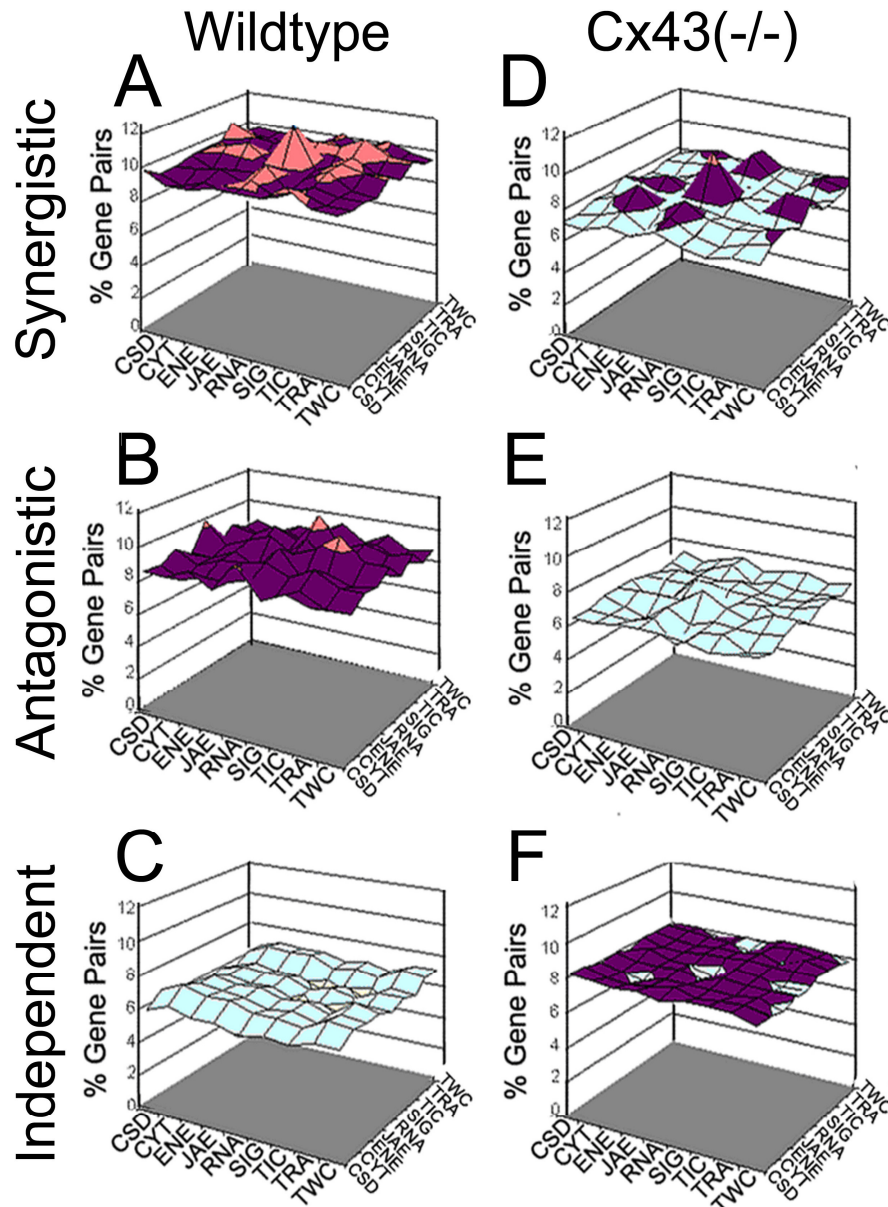
Note that most of the genes antagonistically expressed with Gja1 in wildtype brain were up-regulated in the Gja1 null brain (lower-right corner), most of the synergistically expressed genes with Gja1 in wildtype brain were down-regulated in Gja1 null brain (upper-left corner) and practically none of the genes independently expressed with Gja1 in wildtype brain were significantly regulated in Gja1 null brain (central area on the horizontal plane).

Spray & Iacobas, JMB 2007



Role of Cx43 in intercoordinated gene cohorts

cohort or functional



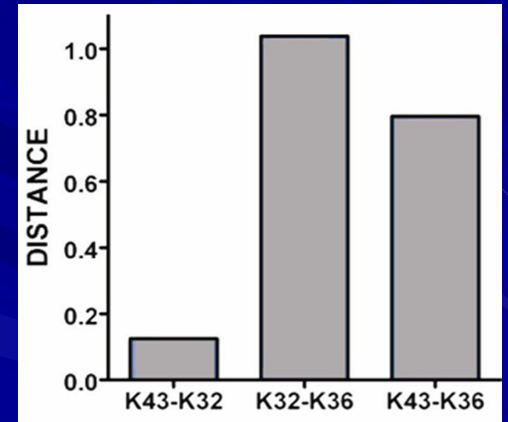
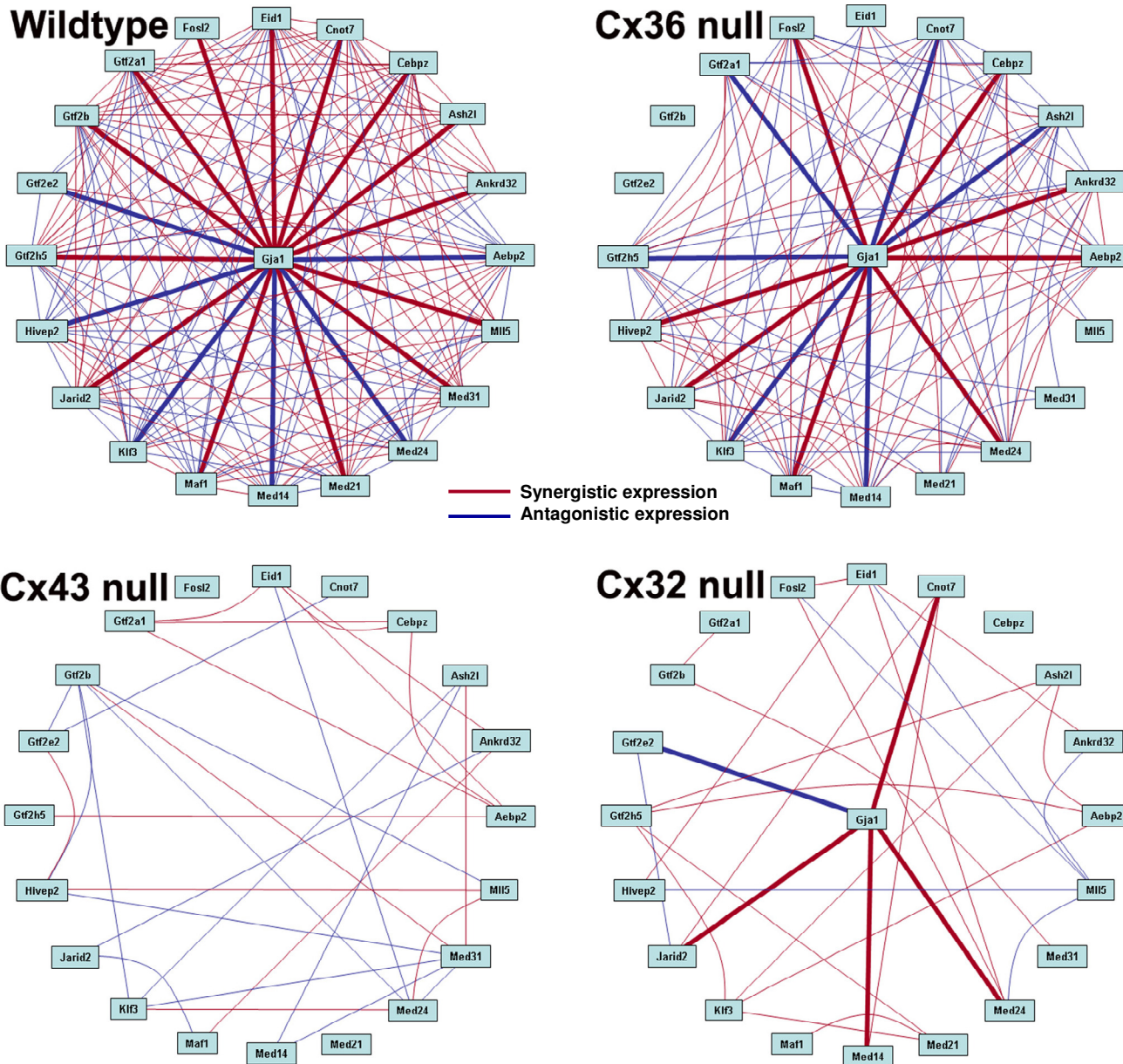
Percentages of synergistically, antagonistically and independently expressed gene pairs within each pair of gene cohorts in brain of neonatal wildtype and *Gja1*^{-/-} mouse

Note the uniformity of synergism, antagonism and independence among pairs of functional categories in both wildtype and *Gja1*^{-/-} brains, the robust reduction of synergism and antagonism for all cohort pairs and the increase of independence among pairs of functional categories in the *Cx43*^{-/-} brain.

CSD = Cell cycle, shape, differentiation, death,
CYT = cytoskeleton,
ENE = Energy metabolism,
JAE = Cell junction, Adhesion, Extracellular matrix,
RNA = RNA Processing,
SIG = Cell Signaling,
TIC = transport of small molecules and ions into the cells,
TRA = Transcription,
TWC = transport of ions/molecules within the cells,
UNK = function not yet assigned.

Iacobas et al, Genomics 2007,

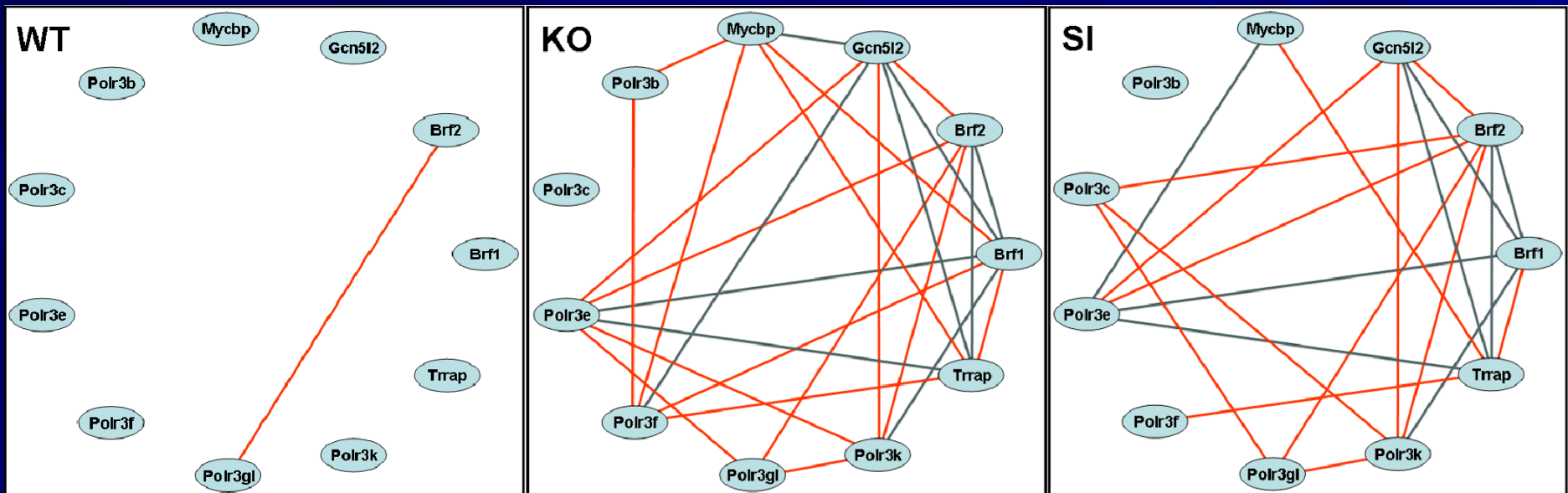
Expressome remodeling in Cx null brains



Iacobas et al., SFN 2008

Part of the Cx43 expressome (20 transcription factors) in the WT brain and its alterations when Cx43, Cx36 or Cx32 are disrupted. Note the substantial alterations and similarities between Cx43 null and Cx32 null brains, and the significantly smaller effect in Cx36 null brain, supporting the idea of the **pan-glial transcriptomic continuity in the brain, independent of the neuronal transcriptomic network.**

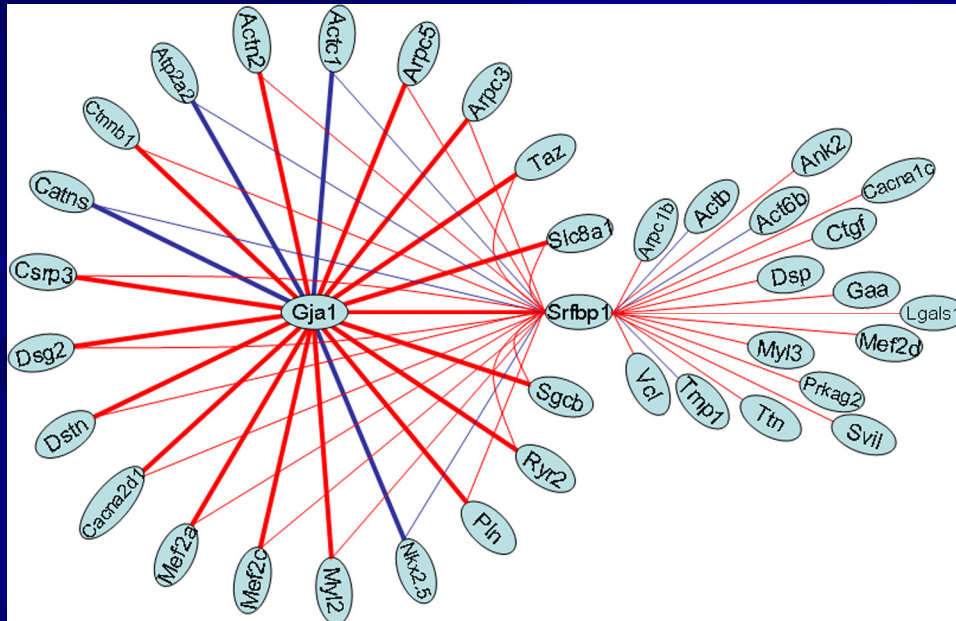
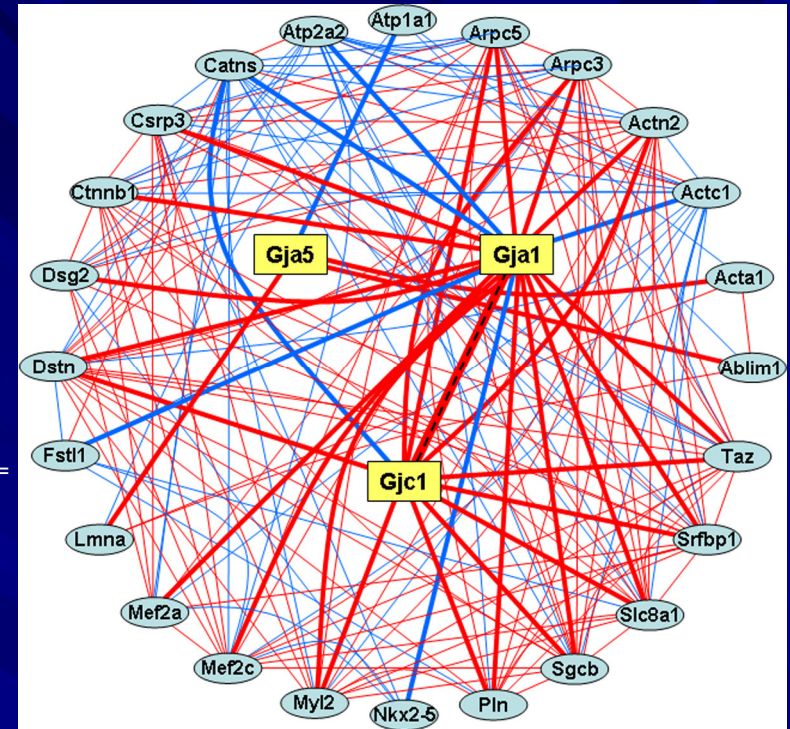
Intercoordonarea genelor din clusterul polimerazei III in astrocitele corticale normale si deficitare de Cx43



Remodeling of gene interlinkages. Note the lack of interlinkage between the genes in WT genotype and their presence in SI and KO. Of note is the similar remodeling of gene interlinkages within the polymerase III cluster induced by knockdown and knockout. Red lines indicate significant synergistic expression of the connected genes and blue lines indicate antagonistic expression. Genes: **Brf1** = BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB (*S. cerevisiae*), **Brf2** = BRF2, subunit of RNA polymerase III transcription initiation factor, BRF1-like, **Gcn512** = GCN5 general control of amino acid synthesis-like 2 (yeast), **Mycbp** = c-myc binding protein, **Polr3b** = polymerase (RNA) III (DNA directed) polypeptide B, **Polr3c** = polymerase (RNA) III (DNA directed) polypeptide C, **Polr3e** = polymerase (RNA) III (DNA directed) polypeptide E, **Polr3f** = polymerase (RNA) III (DNA directed) polypeptide F, **Polr3gl** = polymerase (RNA) III (DNA directed) polypeptide G like, **Polr3k** = polymerase (RNA) III (DNA directed) polypeptide K, **Trrap** = transformation/transcription domain-associated protein.

Coordonarea de catre connexine a genelor reglatoare ale ritmului cardiac in cardiomiocitele HL-1.

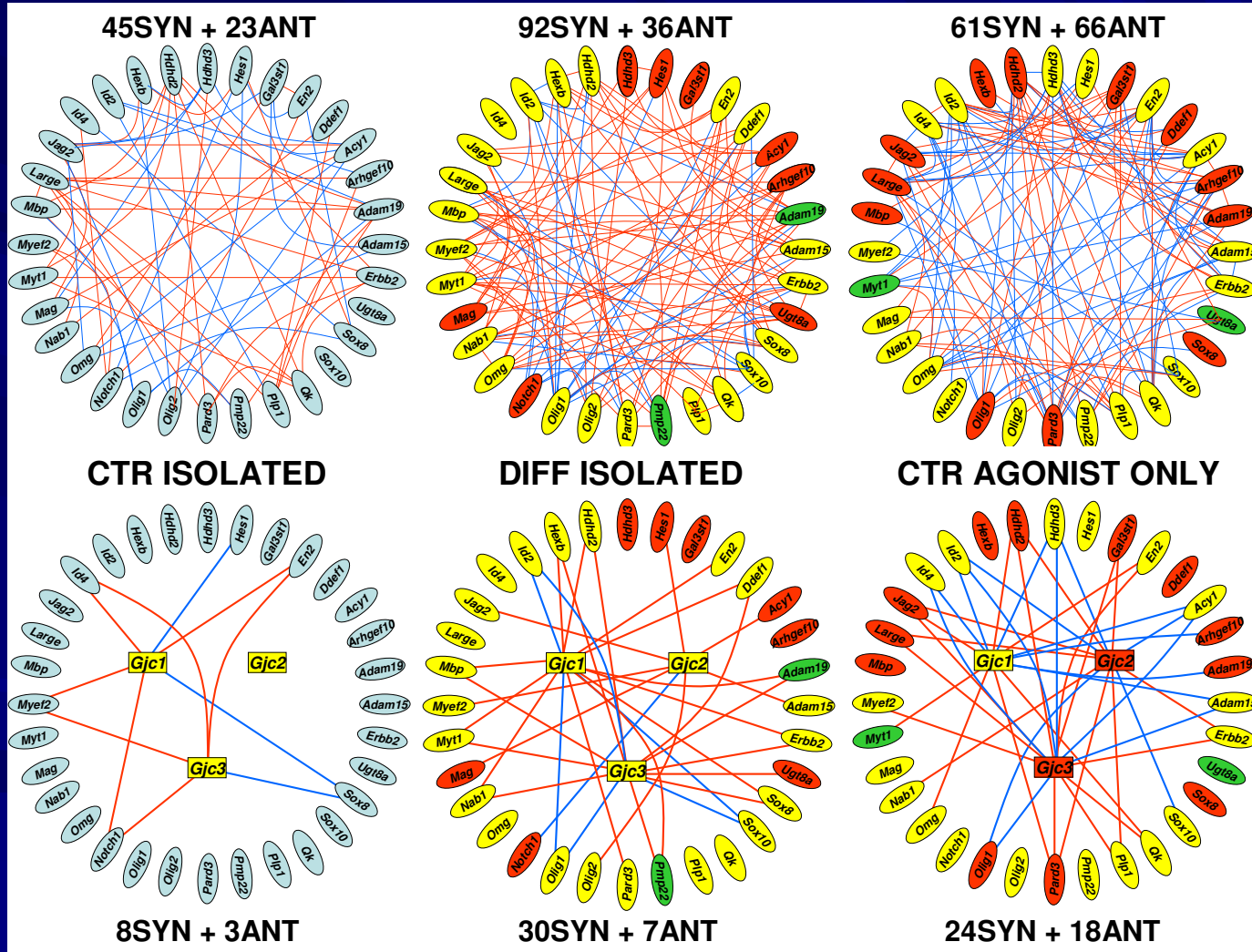
Red and blue colors of the lines indicate synergistic and antagonistic expression coordination among the connected genes. Thicker lines were used to underline the coordinations with the three connexin genes. **Ablim1** = Actin-binding LIM protein 1, **Acta1** = Actin, alpha 1, skeletal muscle, **Actc1** = Actin, alpha, cardiac, **Actn2** = Actinin alpha 2, **Arpc3** = Actin related protein 2/3 complex, subunit 3, **Arpc5** = Actin related protein 2/3 complex, subunit 5, **Atp1a1** = ATPase, Na+/K+ transporting, alpha 1 polypeptide, **Atp2a2** = ATPase, Ca++ transporting, cardiac muscle, slow twitch 2, **Catns** = Catenin src, **Csrp3** = cysteine and glycine-rich protein 3, **Ctnnb1** = Catenin (cadherin associated protein), beta 1 **Dsg2** = Desmoglein 2, **Dstn** = Destrin, **Fstl1** = Follistatin-like 1, **Lmna** Lamin A, transcript variant 1, **Mef2a** = Myocyte enhancer factor 2A, transcript variant 1, **Mef2c** = Myocyte enhancer factor 2C, **My12** = myosin, light polypeptide 2, regulatory, cardiac, slow, **Nkx2.5** = NK2 transcription factor related, locus 5 (Drosophila), **Pln** = Phospholamban, **Sgcb** = sarcoglycan, beta (dystrophin-associated glycoprotein), **Slc8a1** = solute carrier family 8 (sodium/calcium exchanger) member 1, **Srfbp1** = Serum response factor binding protein 1, **Taz** = tafazzin. The HRD genes illustrated in this figure were selected only from literature before applying the very computer intensive PGA.



Intercoordonarea expresiilor de reglare a ritmului cardiac de catre Cx43 si Serum response factor binding protein1 (Srfbp1) in celule HL-1.

Arpc1b = Actin related protein 2/3 complex, subunit 1B, **Arpc5** = Actin related protein 2/3 complex, subunit 5, **Actb** = Actin, beta, cytoplasmic, **Act16b** = Actin-like 6B, **Ank2** = Ankyrin 2, brain, **Cacna1c** = Calcium channel, voltage-dependent, L type, alpha 1C subunit, **Mef2d** = Myocyte enhancer factor 2D, **Ryr2** = Ryanodine receptor 2, cardiac.

Rolul Cx29, Cx45 si Cx47 in coordonarea genelor de mielinizare



Concluzii

- Ablatia oricarei connexine altereaza nivelul de exprimare, variabilitatea si coordonarea a > 10% din genomul investigat
- Genele alterate sunt localizate in toti cromozomii si codifica proteine din toate categoriile functionale
- Exprimarea coordonata cu Cx in tesutul de tip salbatic prezice cu acuratete remarcabila alterarea exprimarii in tesutul knockout si knockdown de Cx
- Exista gene ale caror profile de coordinatie sunt similare sau opuse profilelor conexinelor in tesuturile de tip salbatic, profile care se modifica semnificativ in tesuturile KO sau KD, sau expuse la conditii extreme. Manipularea exprimarii unor astfel de gene "see-saw" (balansoar) poate in principiu sa readuca transcriptomul in limite normale.
- **Conexinele actioneaza ca noduri transcriptomice, controland numeroase procese vitale**