Tumor exosomes as circulating biomarkers?

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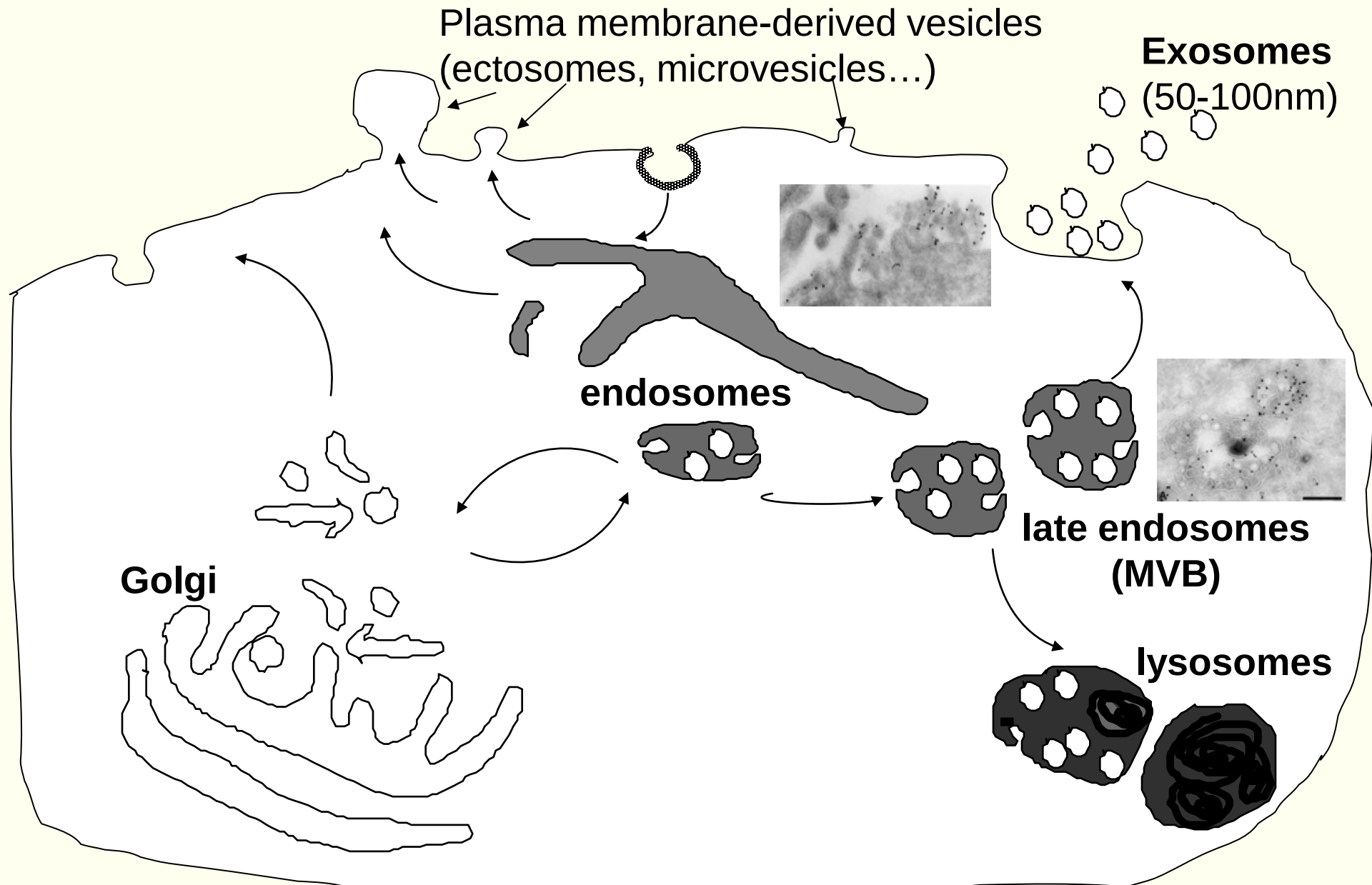
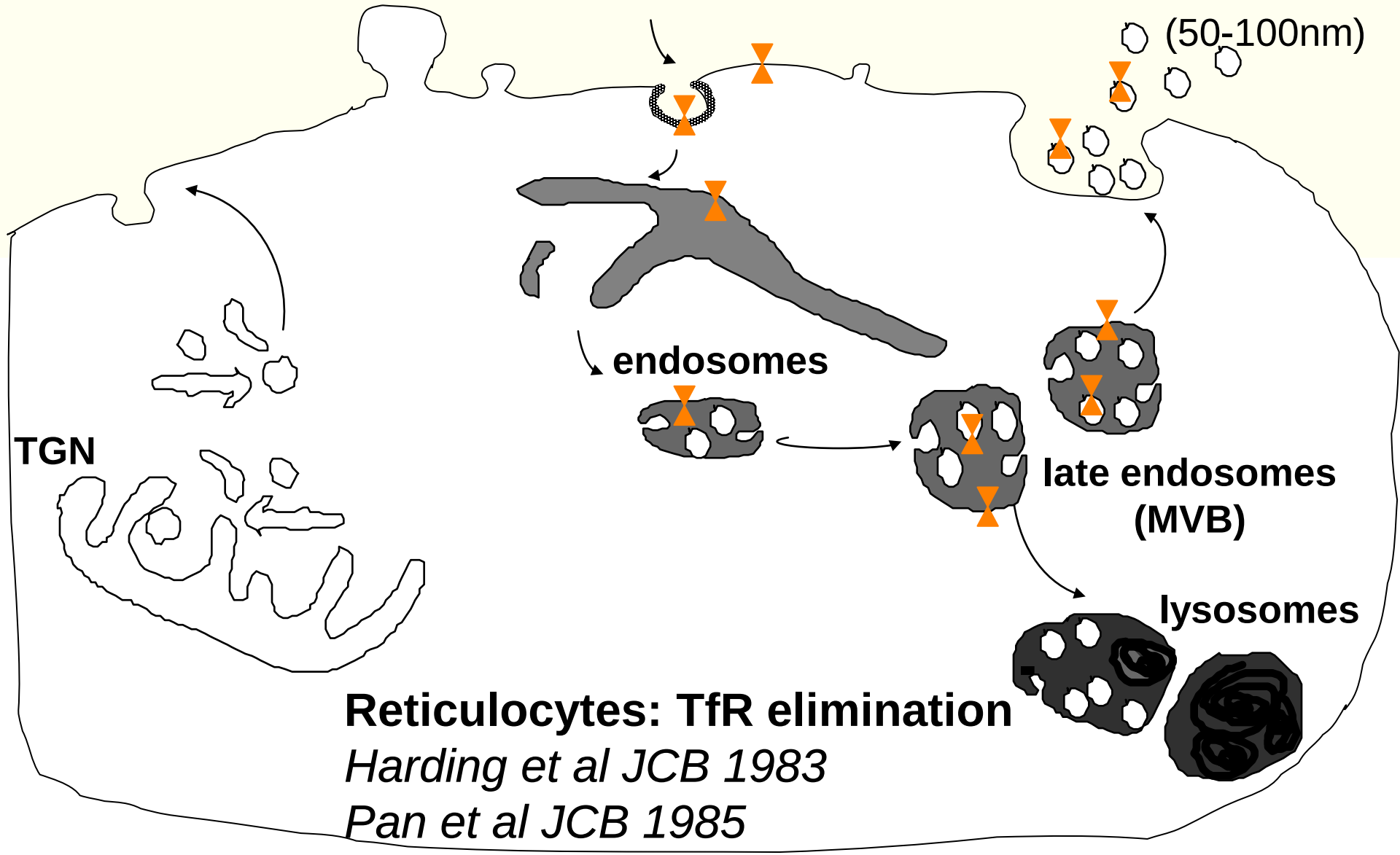
Institut Curie, INSERM U932, « Immunity and Cancer », Paris

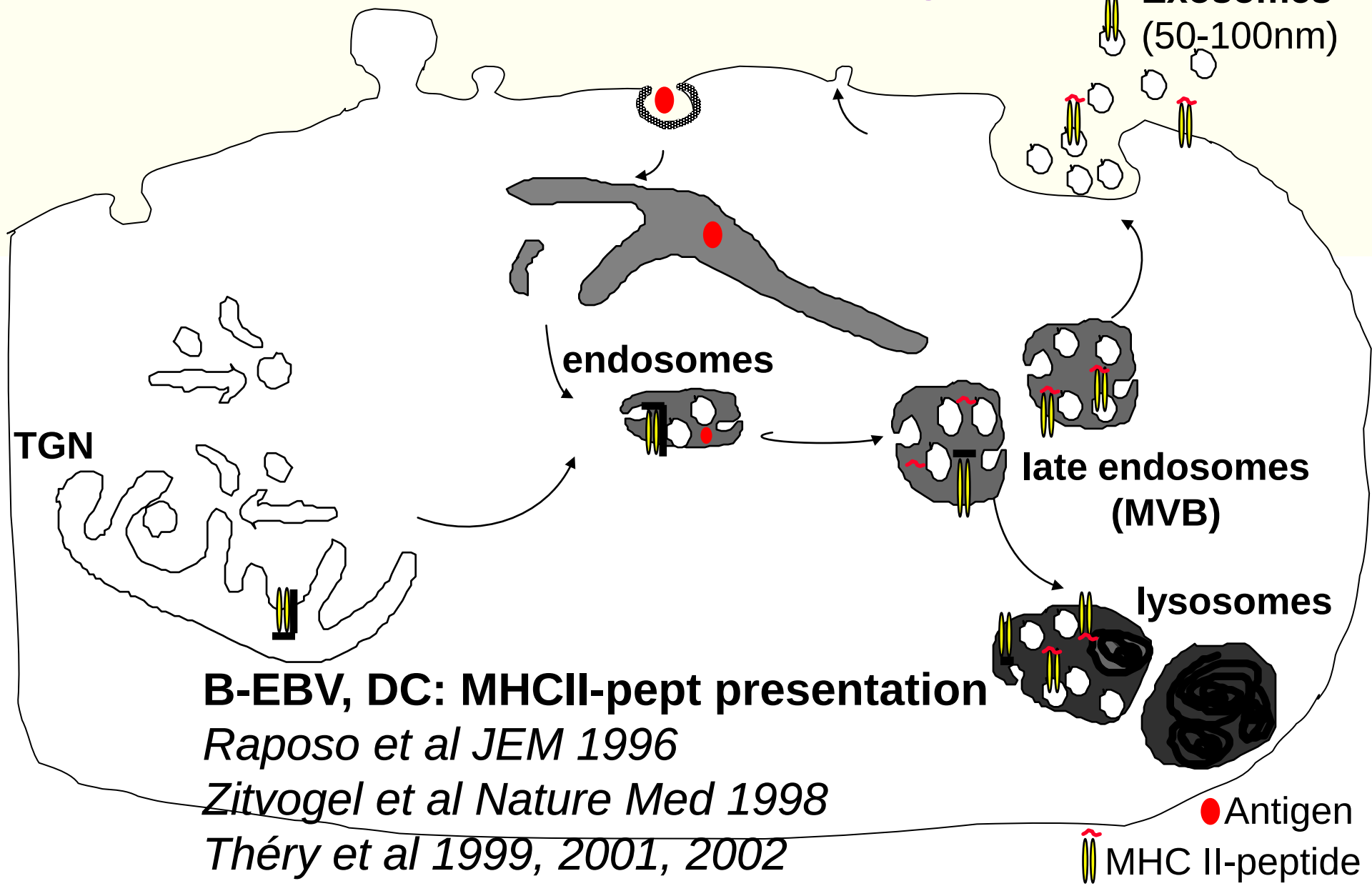
exosomes : a subtype of secreted membrane vesicles originating from late endosomes



exosomes : a means for reticulocytes to eliminate unwanted proteins

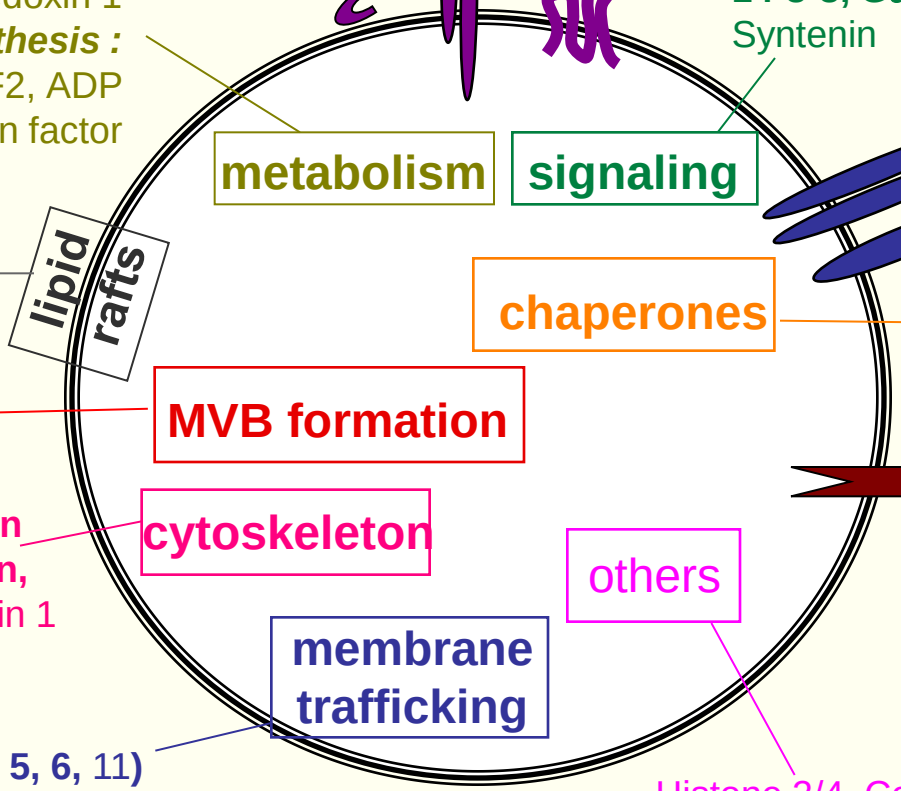


exosomes : a means for antigen-presenting cells to communicate immune signals



protein composition of a « canonical » exosome

generally purified by
differential ultracentrifugation
(final: 100,000g)



Targeting / adhesion

Integrins

Tetraspanins (CD63, CD81, CD9)

MFGE8

Enzymes:
GAPDH, Pyruvate kinase, aEnolase, Phosphoglycerate kinase 1, Peroxiredoxin 1

Protein synthesis :
Ef1a1, eEF2, ADP ribosylation factor

14-3-3, Ga, Syntenin

T cell stimulating molecules

MHC II

MHC I

metabolism

signaling

Flottilin-1

lipid rafts

chaperones

Hsc70, Hsp90
cyclophilin A

Alix, Tsg101, **clathrin,** Ubiquitin

MVB formation

Transmembrane molecules

Actin, Tubulin
Ezrin, Moesin,
Radixin, Cofilin 1
myosin

cytoskeleton

others

membrane trafficking

CD26/Dipeptidylpeptidase
CD13/Aminopeptidase
ATPase Na+/K+ transporting
CD98

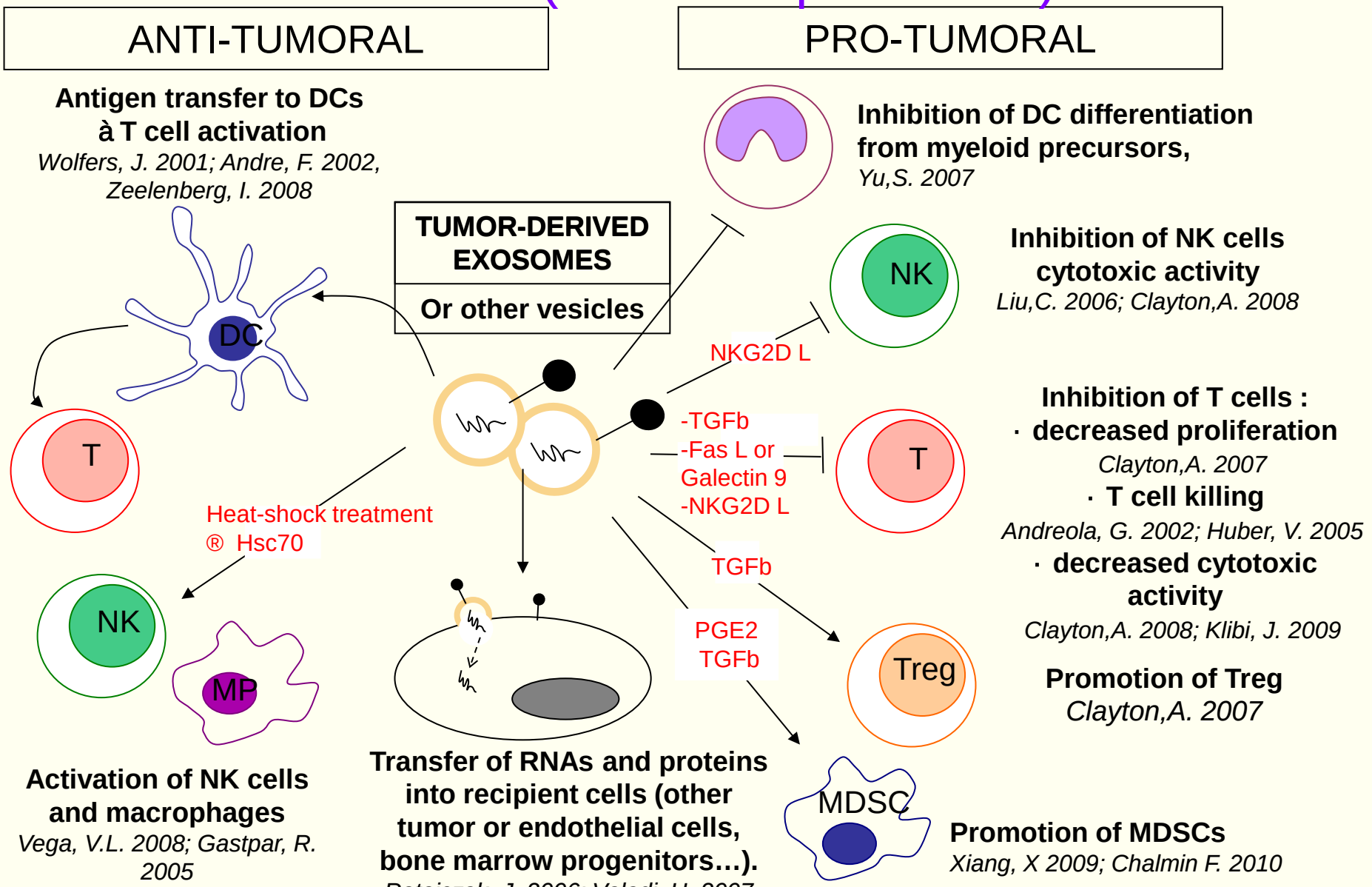
Annexins (1**, 2, 4, 5, 6,** 11**)**
Rab (2**, 7,** 10, 11B**),**
Rab GDI, Rap1B

Histone 2/4, Complement factor**,** MVP, ferritin**,** atypically secreted proteins

compilation of proteomic studies 1999-2010:
http://microvesicles.org

Chaput and Théry, Semin Immunopathol. 2011

Tumors secrete exosomes with various proposed functions (in vitro experiments)



Bobrie et al, Traffic 2011

Proposed use of tumor exosomes (or other membrane vesicles) and/or their RNA as circulating biomarkers in cancer

Exosomes in biofluids:
Tumor ascites *(Andre 2002)*, blood (*Caby 2005*), urine (*Pisitkun 2004*), saliva (*Ogawa 2008*)…

Increased amount of membrane vesicles or tumor-derived membrane proteins in blood of cancer patients:
ovarian/endometrial cancer (*Taylor 2002*), glioblastoma (*Skog 2008*), nasopharyngeal carcinoma (*Klibi 2009*), melanoma (*Logozzi 2009, Peinado 2012*)

mRNA and miRNA in exosomes purified from normal cells (*Valadi 2007*) and from blood including in cancer patients (*Skog 2008*)

miRNA signature in circulating exosomes from cancer patients ?

Exosomes / Other secreted vesicles / miRNA as circulating cancer biomarkers

miRNA signature in circulating exosomes from cancer patients ?

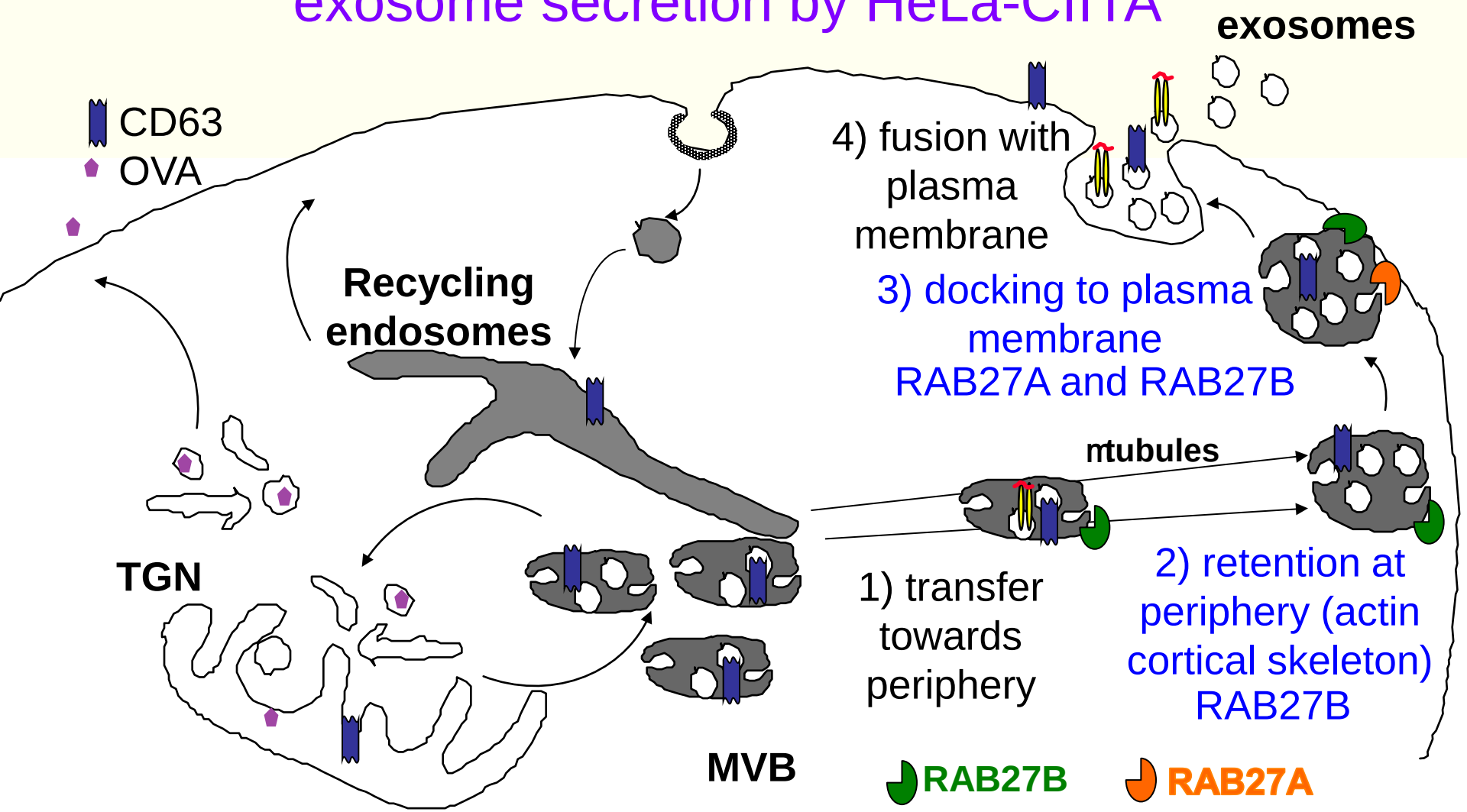
Circulating miRNA can be associated to exosomes/membrane vesicles, but also to protein (*Arroyo 2011*) or lipoprotein (*Vickers 2011*) complexes

Vesicles in blood circulation have been studied for 30 years: microparticles, shed membrane vesicles, microvesicles, exosomes…

Question: Are all these vesicles identical ?

Approach: to identify intracellular molecular mechanisms of extracellular vesicle secretion

Screening a library of shRNA inhibiting expression of RAB genes : a role for RAB27A and RAB27B in exosome secretion by HeLa-CIITA



Ostrowski et al, Nature Cell Biol 2010

Conclusions

Identification of RAB27A/B as crucial players in spontaneous secretion of exosomes by HeLa-CIITA cells

RAB27A/B are known to be involved in regulated exocytosis of secretory granules and lysosome-like compartments = compartments of endosomal (or mixed) nature

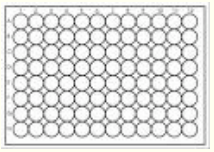
new experimental evidence of endosomal origin of exosomes

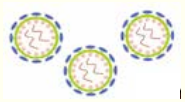
use of these results for ***in vivo*** studies of exosome functions:

role of Rab27a/b in exosome secretion by other cells?
analysis of mouse tumor cells

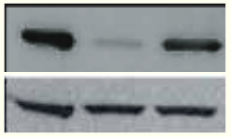
Effect of inhibition of Rab27a or Rab27b on exosome secretion by mouse mammary tumor cells (4T1, TS/A)

Cell seading → 24h → Infection with lentiviruses expressing shRNA 27a or 27b → 36h → Selection (puromycin) → Expansion





shRNA Scr 27a 27b



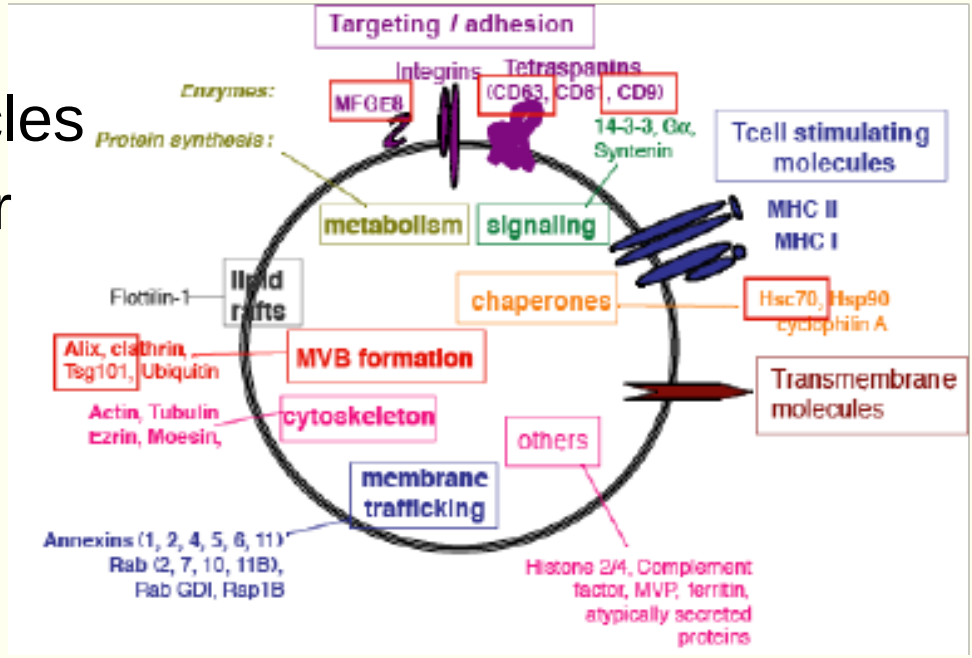
Rab27a

actin

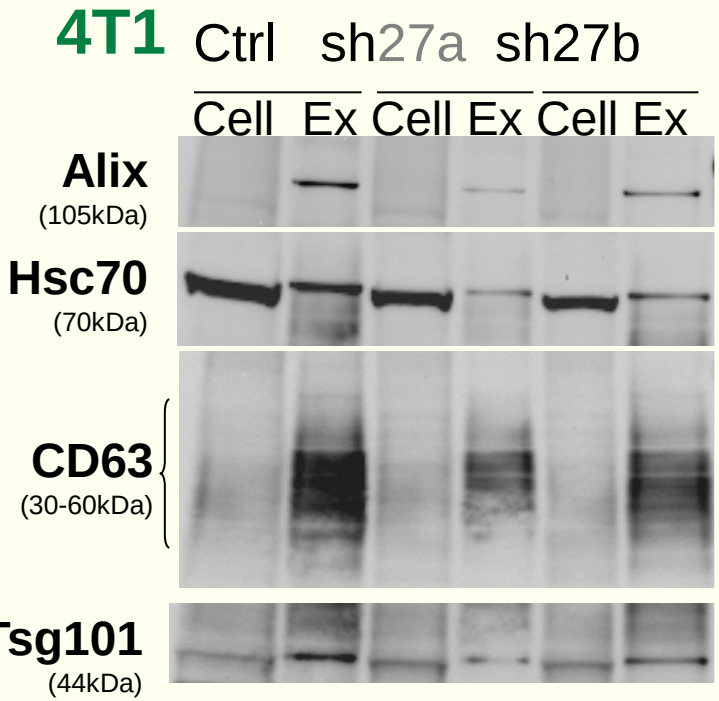
Purification of exosomes (10,000g then 100,000g) from supernatant of Rab27-invalidated cells :

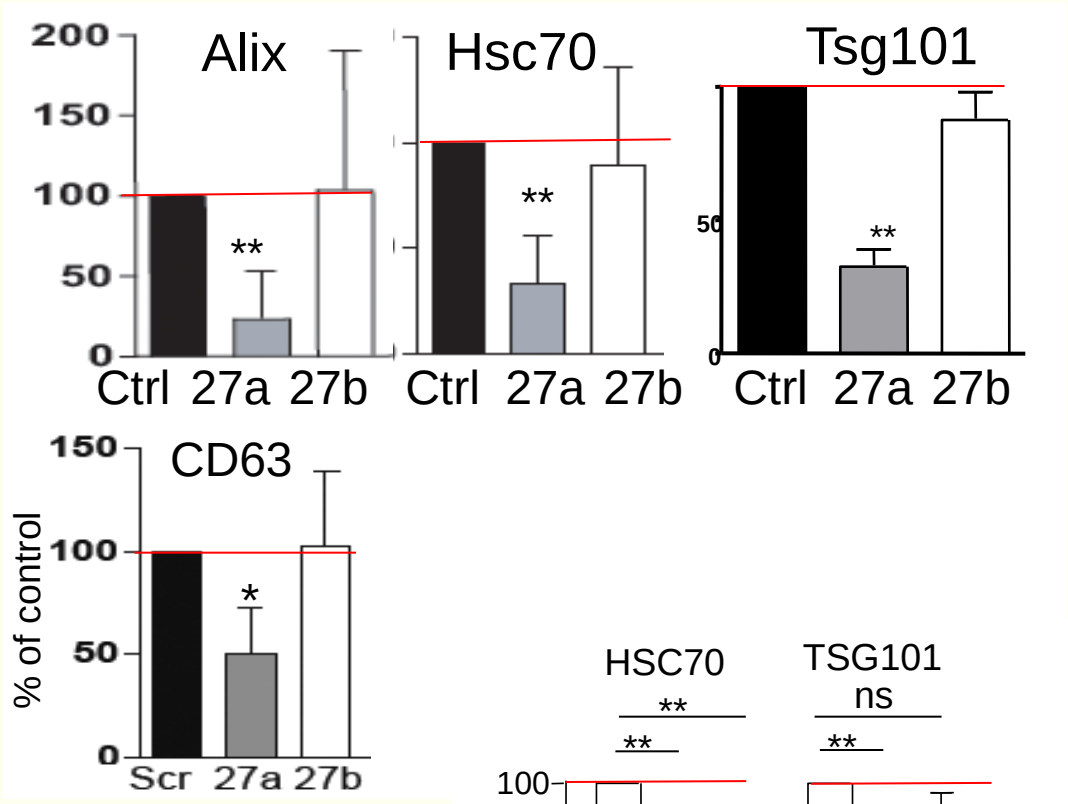
Loading on SDS-PAGE of vesicles secreted by a given cell number

Analysis by Western blot of protein markers



inhibition of Rab27a (but not Rab27b) decreases secretion of “endosomal” exosome markers by 4T1

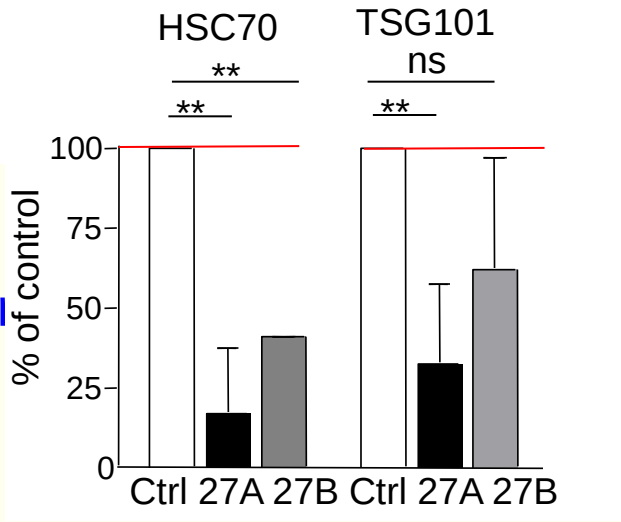




Cell= total proteins from 10^4 cells

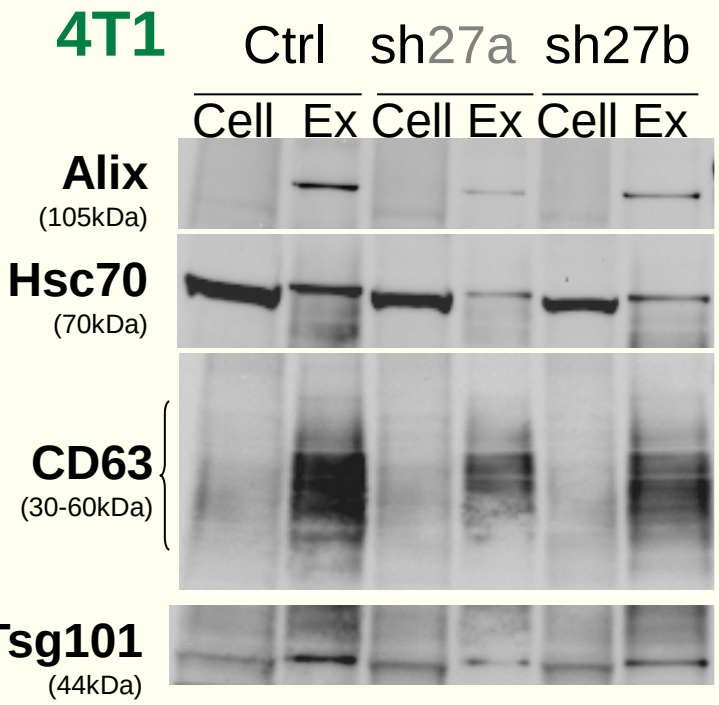
Ex = Exosomes (100,000g) secreted by 15 10^6 cells

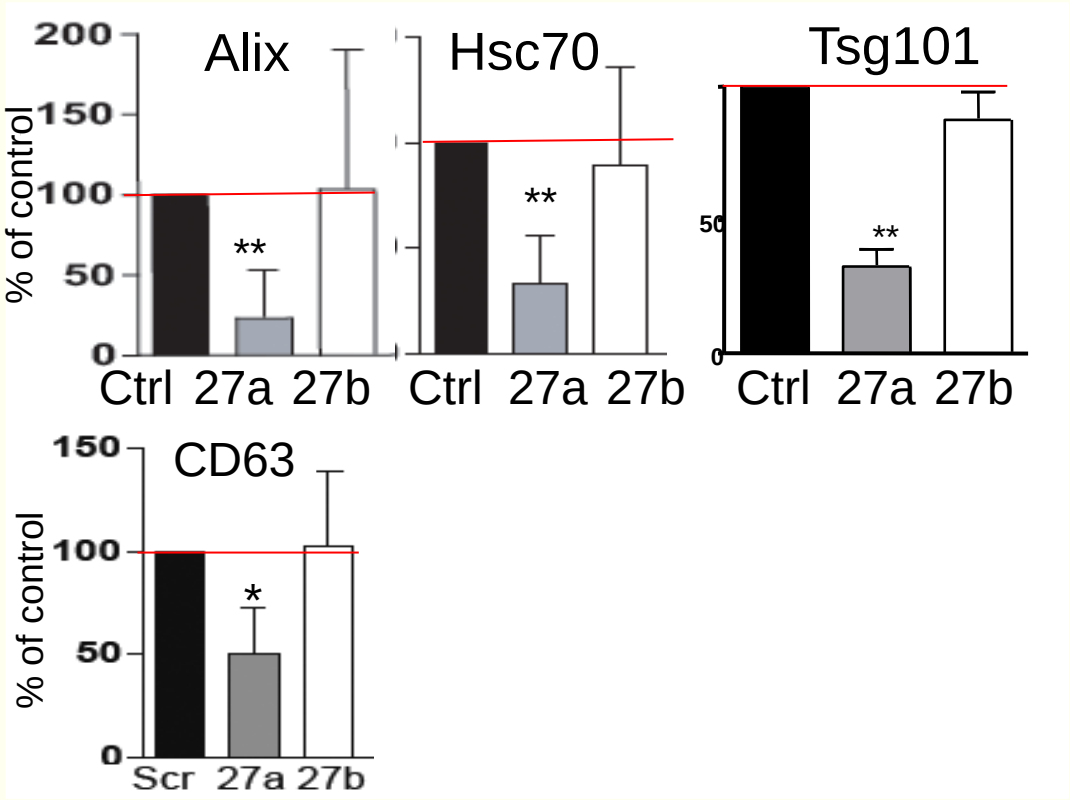
HeLa (*Ostrowski et al NCB 2010*): sh to either RAB27A or RAB27B decrease secretion of « endosomal » markers

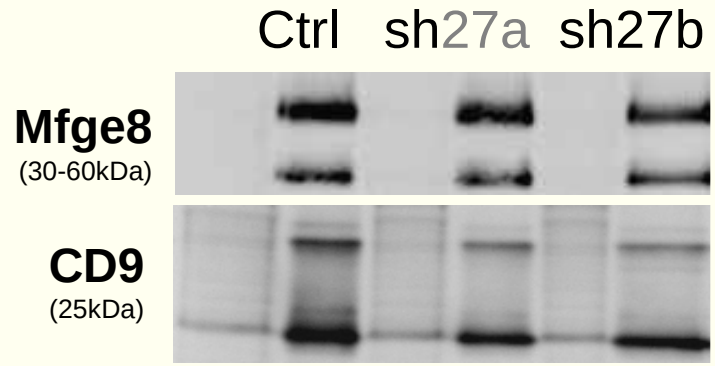


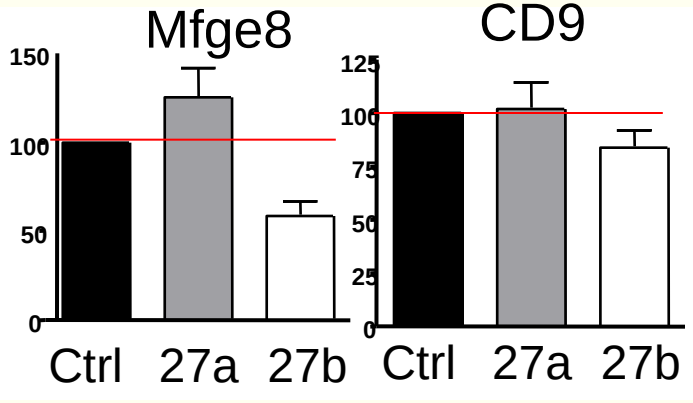
Bobrie et al, Cancer Res 2012

inhibition of Rab27a (or Rab27b) does not decrease secretion of CD9 and Mfge8 in mouse exosomes



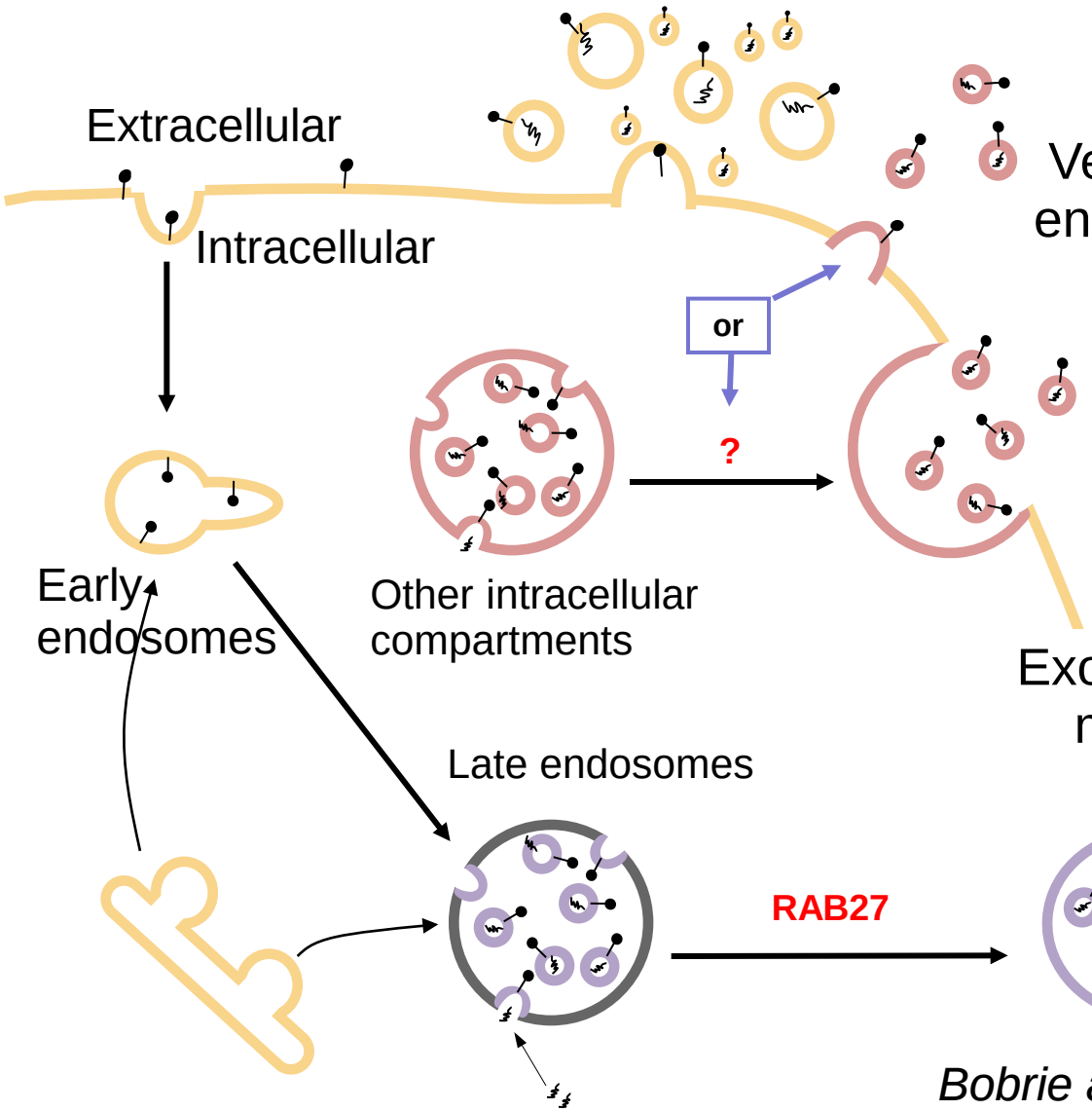






Bobrie, Colombo et al, J Extracell Vesicles 2012

Diverse subpopulations of vesicles secreted by different intracellular mechanisms are co-purified by the currently used protocols



Vesicles co-purified with late endosome-derived exosomes (CD9, Mfge8)

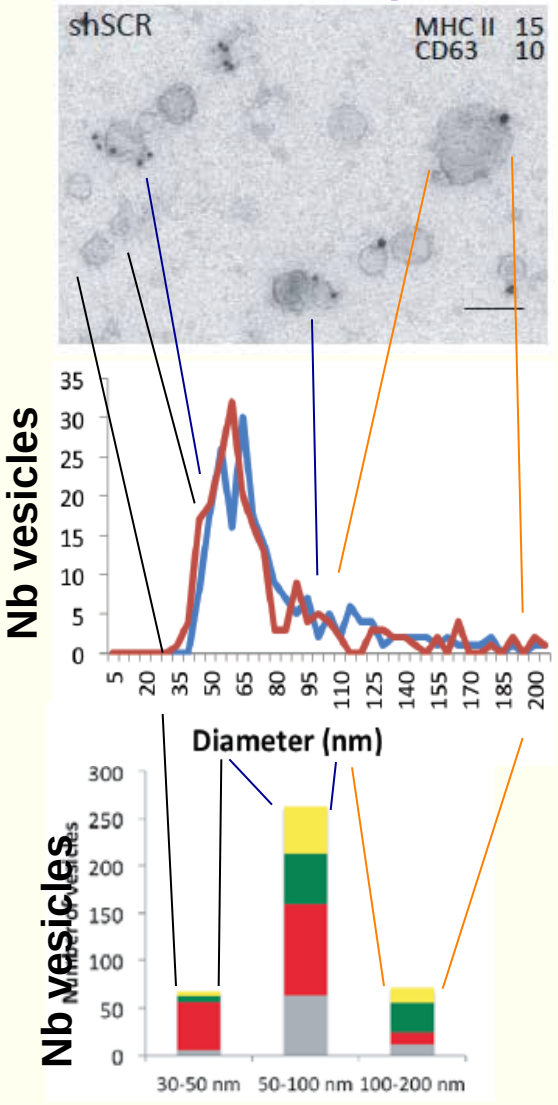
different functions ?

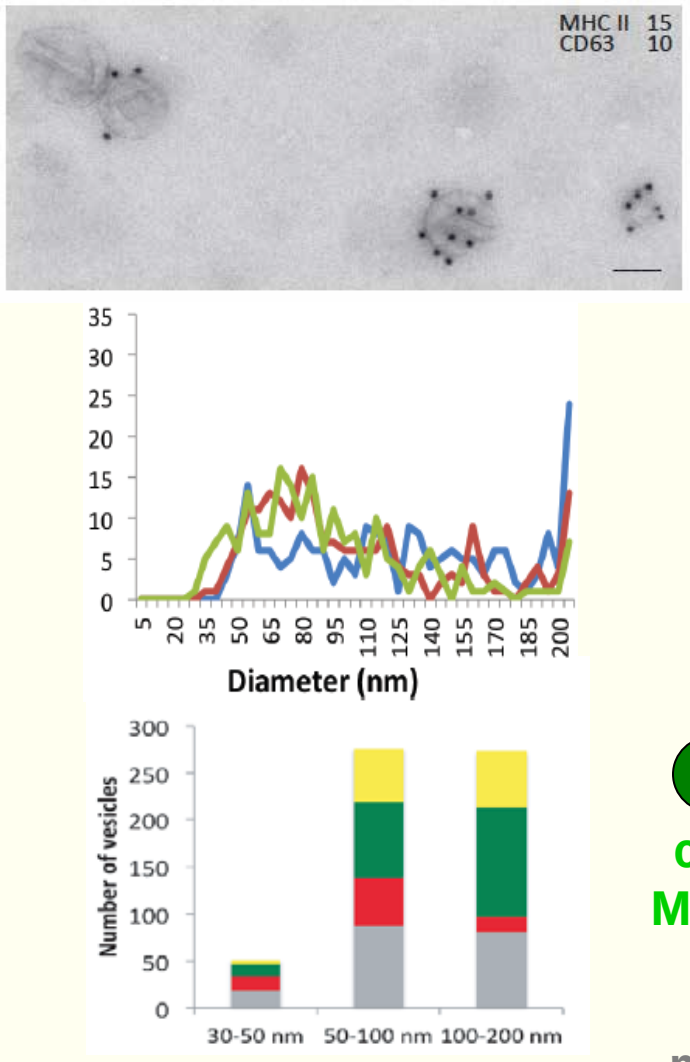
Exosomes bearing late endosome markers (CD63, Tsg101, Alix, Hsc70) (+CD9, Mfge8)

Bobrie, Colombo et al, JEV 2012
Bobrie and Théry, Biochem Soc Trans 2013

Vesicles purified from a human tumor (HeLa) and human Dendritic Cells display very different size distribution and protein composition (CD63/MHC II)

HeLa-CIITA





Human moDCs

only MHC II

CD63 + MHC II

none

only CD63

Colombo et al, J Cell Sci (in press)

New Challenges for use of exosomes in biological fluids of cancer patients as diagnostic or prognostic marker:

Vesicles purified from blood are of various cellular (tumor cells, endothelial cells, immune cells) AND intracellular origins

⟶ What is the precise protein and nucleic acid composition of the different subtypes of vesicles?

⟶ Do some or all (or none!) of them have a value as diagnostic or prognostic marker?

⟶ Are some of them of better efficacy as therapeutic tools?

Need to re-establish purification and characterization of vesicles isolated from biological fluids of patients

INSERM U932, Institut Curie, Paris
(Sebastian AMIGORENA)

"Exosomes and tumor growth"

James Vigneron

Angélique Bobrie

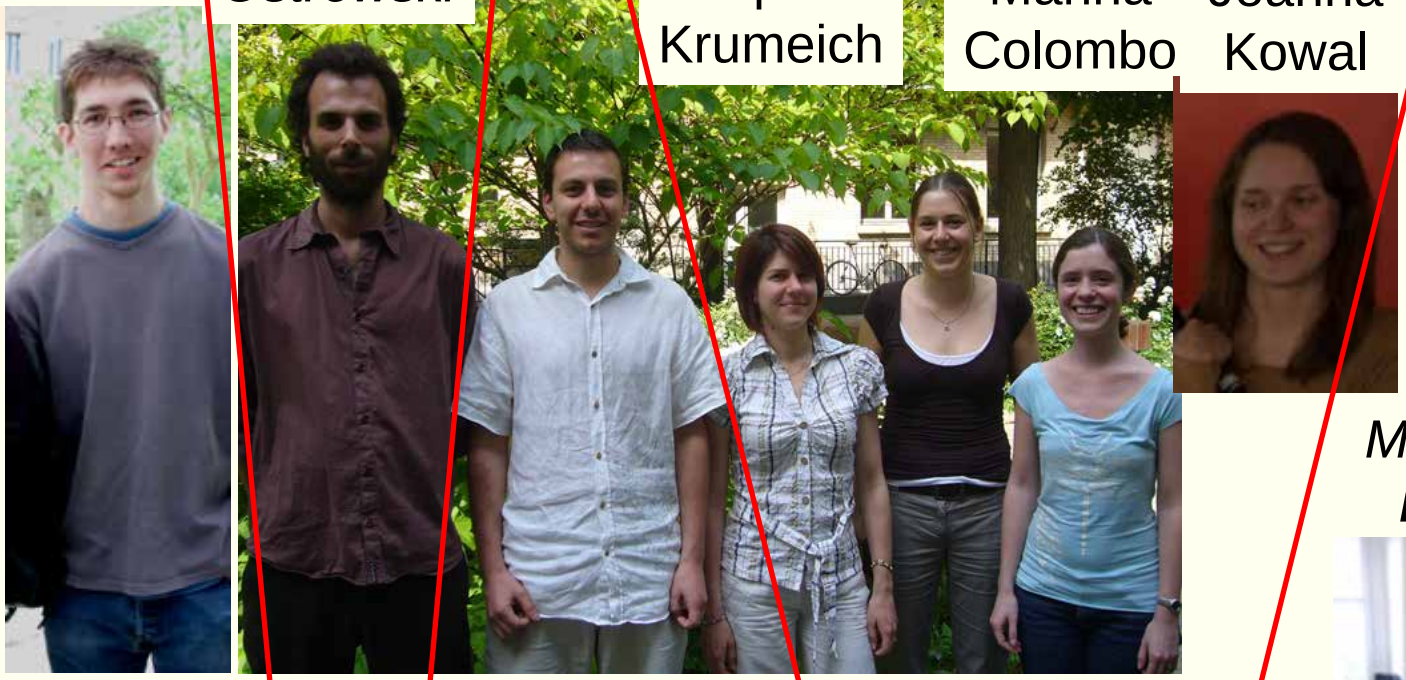
Gaël Sugano

Matias Ostrowski

Sophie Krumeich

Marina Colombo

Joanna Kowal



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CNRS UMR144, Institut Curie, Paris: Graça RAPOSO, Guillaume van NIEL

Institute of Molecular Biology, Lisbonne, Portugal: Luis F.MOITA

Imperial College, London, GB: Chiara RECCHI, Miguel SEABRA

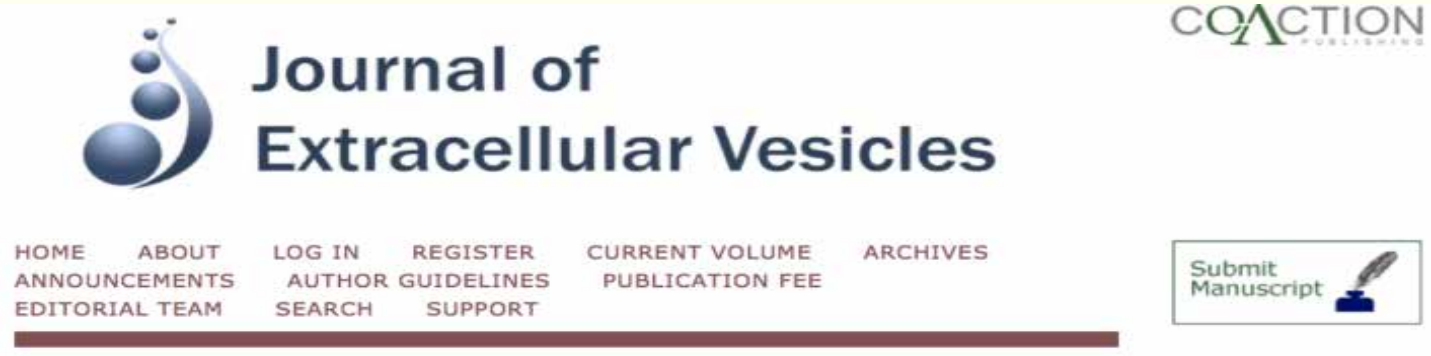
September 2011:
creation of a new International scientific Society dedicated to microvesicles, exosomes, ectosomes and other Extracellular Vesicles

ISEV
INTERNATIONAL SOCIETY FOR EXTRACELLULAR VESICLES

www.isev.org

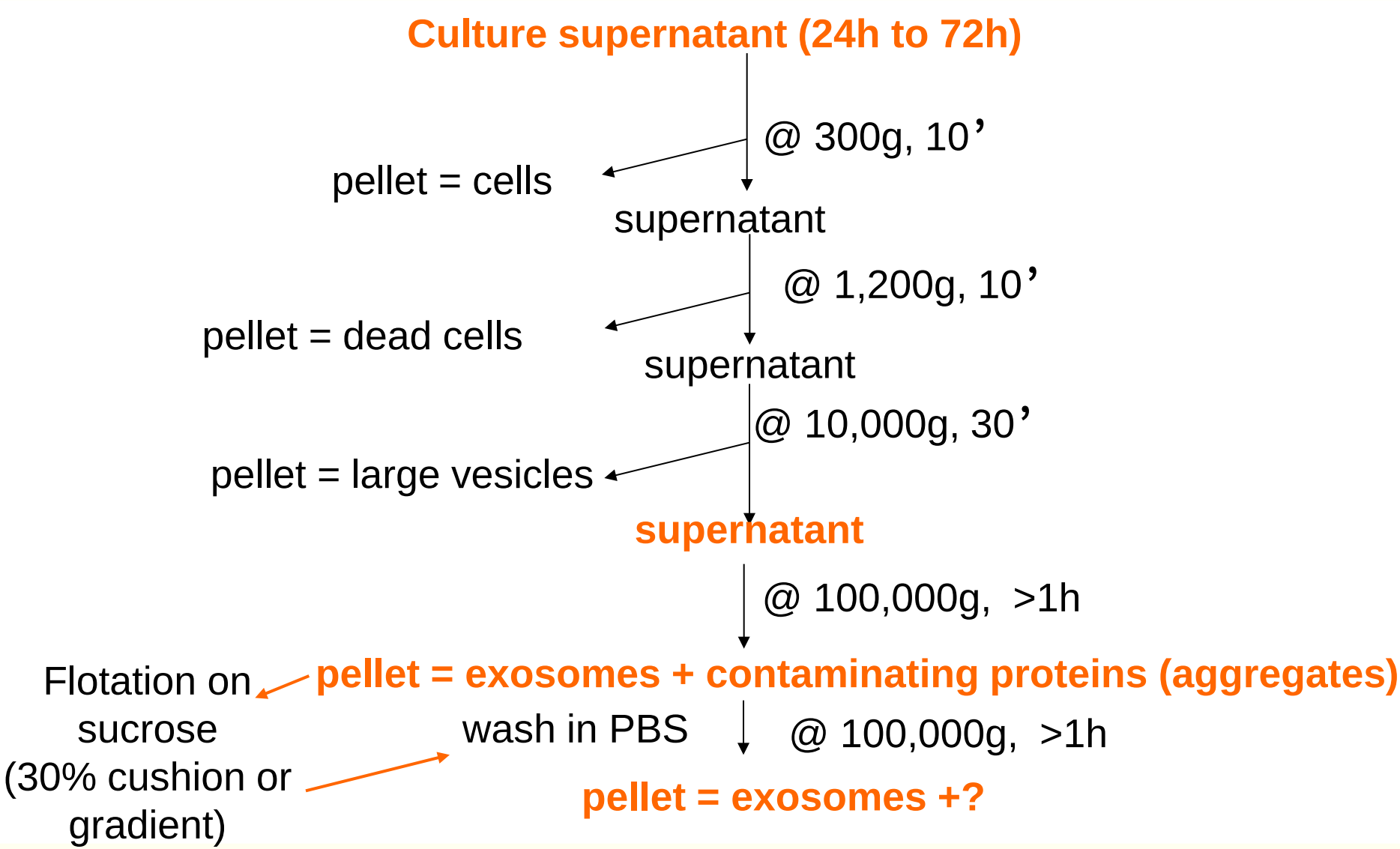
Annual Meeting of ISEV : April 2012 (Göteborg, Sweden), April 2013 (Boston, USA), April-May 2014 (Rotterdam, NL)

Journal launched 16th April 2012 (fully open access):



www.journalofextracellularvesicles.net

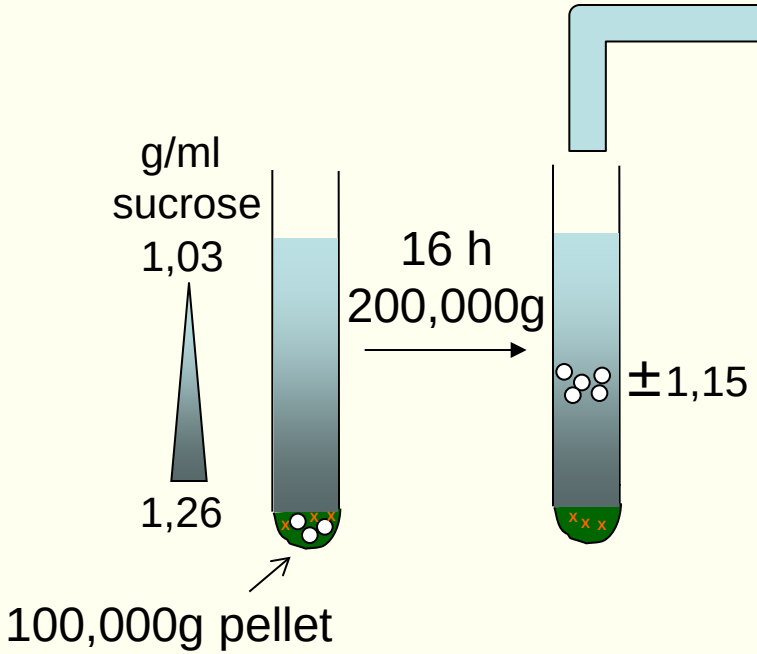
Exosome purification from a cell culture supernatant : differential ultracentrifugation



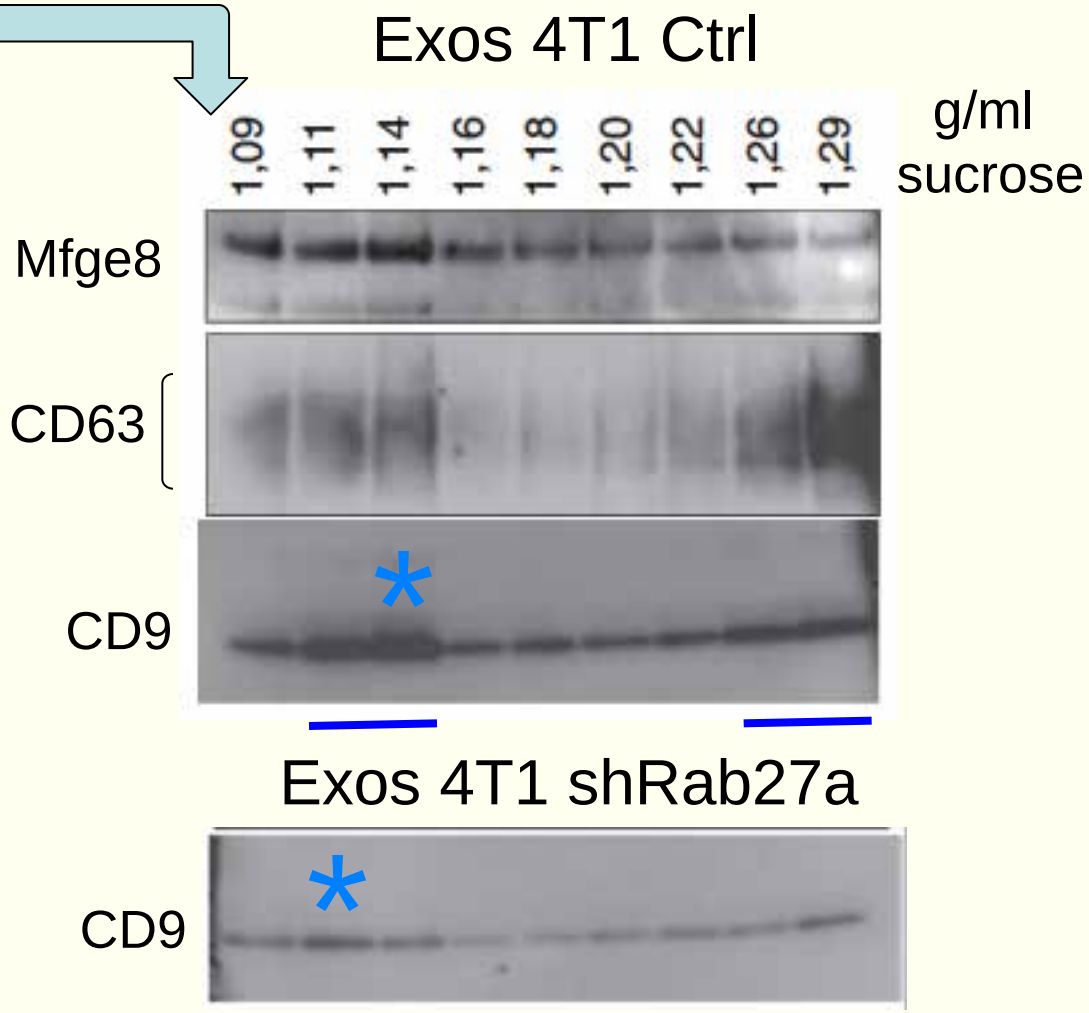
Thery et al, Current Protoc Cell Biol 2006

CD9, CD63 and Mfge8 display subtly different patterns of floatation on sucrose gradients

the 1.14 g/ml fraction is reduced upon Rab27a inhibition



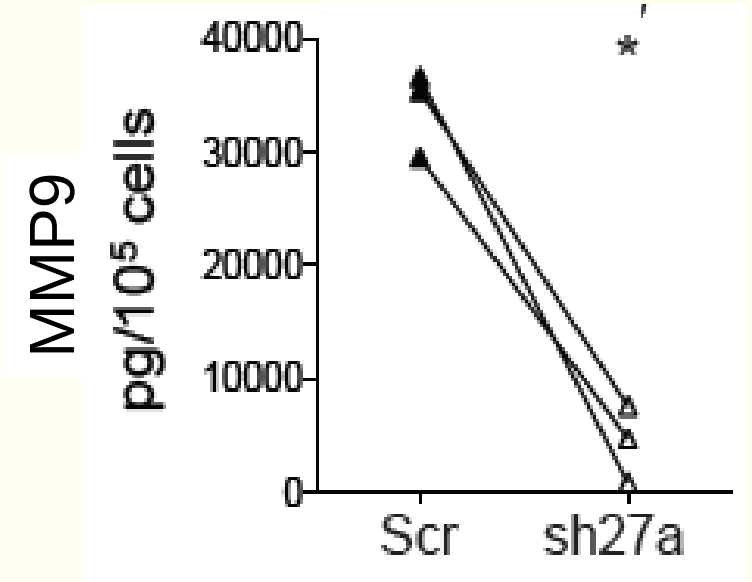
Floatation of exosome pellet
Into a sucrose gradient to
improve purification



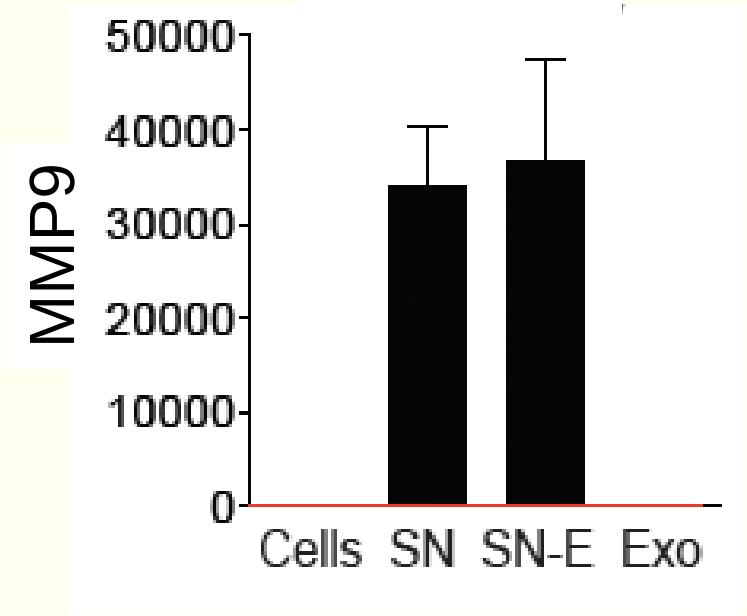
Bobrie, Colombo et al, J Extracellular Vesicles 2012, 1: 18397

Rab27a inhibition decreases exosome-independent secretion of a matrix metalloproteinase (MMP9) by 4T1

MMP9 secretion by 4T1-Scr and 4T1-sh27a

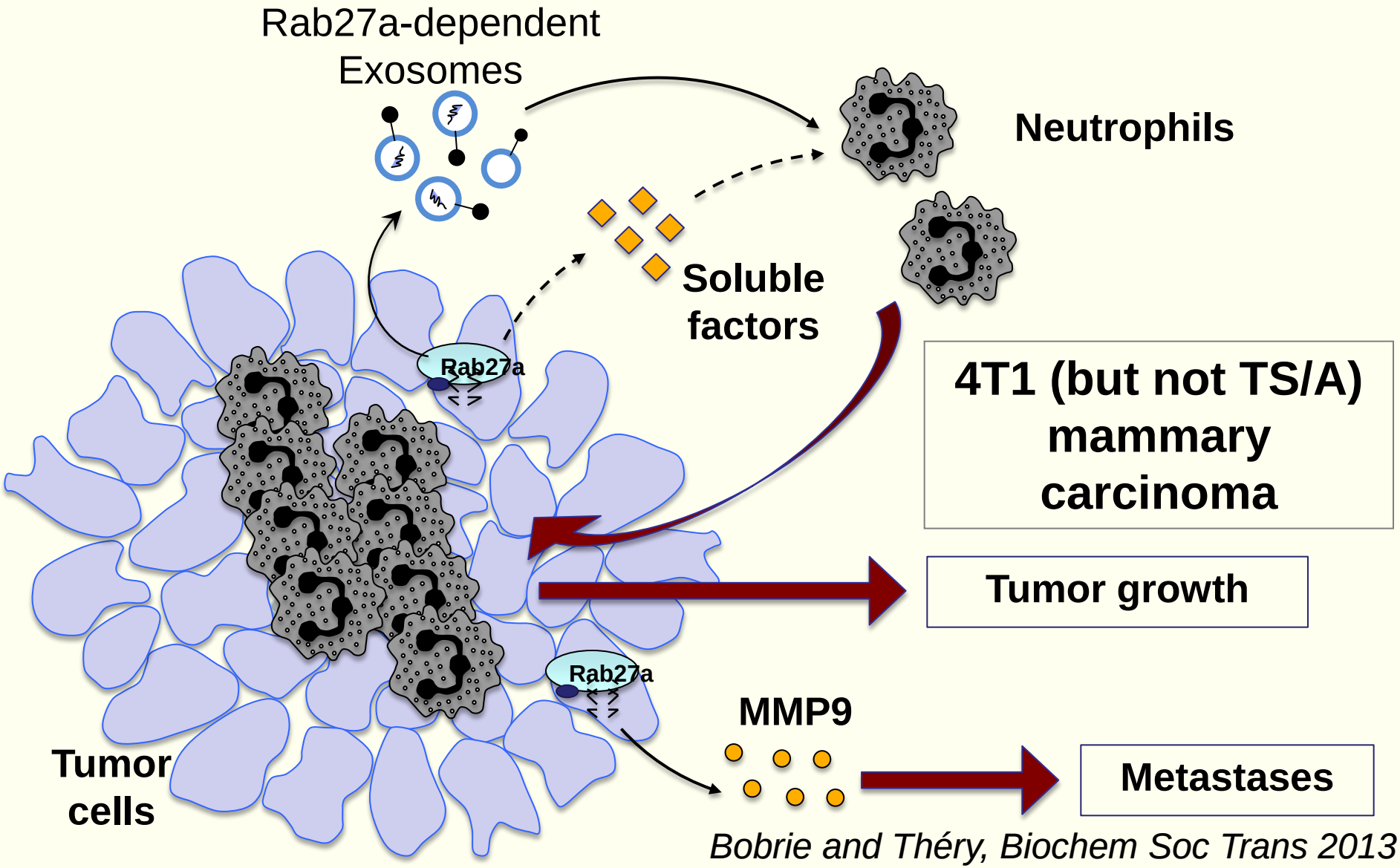


MMP9 secretion in supernatant (SN), SN depleted of exosomes (SN-E) and Exosomes (Exo)



Bobrie et al, Cancer Res 2012

Conclusion: Rab27a promotes exosome-dependent and-independent mechanisms that modify the tumor microenvironment and can promote tumor progression



Bobrie and Théry, Biochem Soc Trans 2013