Opioid genetics
Translational research. What next?

Pål Klepstad

Cecilie Baar

Sonja Andersen

Frank Skorpen

Variations in opioid efficacy? We all know about it.

She needs more morphine

than him

Photos shown with permission

Variable effect from analgesics
Patients respond different to different opioids

SPECIAL ARTICLE

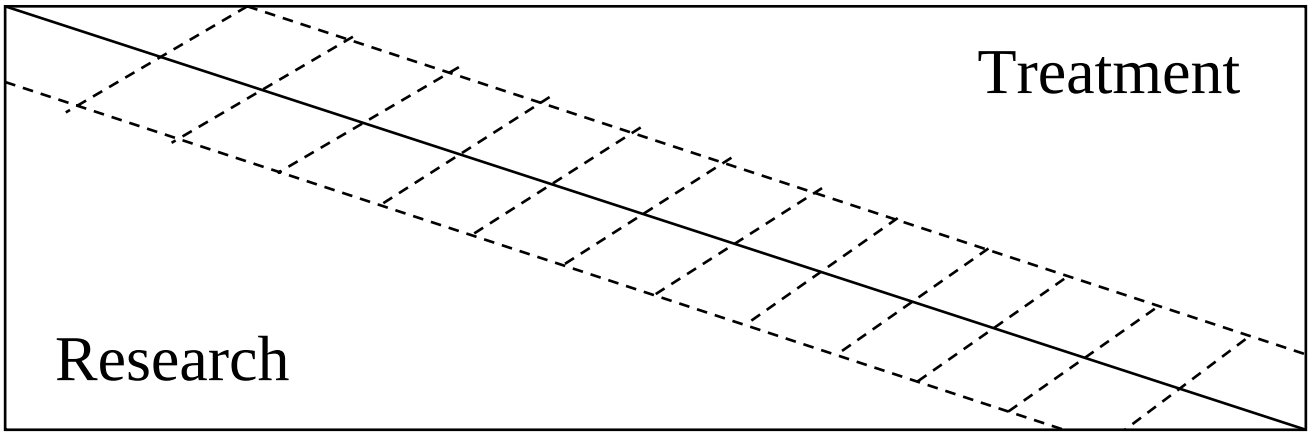
Strategies to Manage the Adverse Effects of Oral Morphine: An Evidence-Based Report

By Nathan Cherny, Carla Ripamonti, Jose Pereira, Carol Davis, Marie Fallon, Henry McQuay, Sebastiano Mercadante, Gavril Pasternak, and Vittorio Ventafridda for the Expert Working Group of the European Association of Palliative Care Network

If side effects persist, the clinician should consider options of symptomatic management of the adverse effect, opioid rotation, or switching route of systemic administration.

J Clin Oncol 2001

Research and treatment



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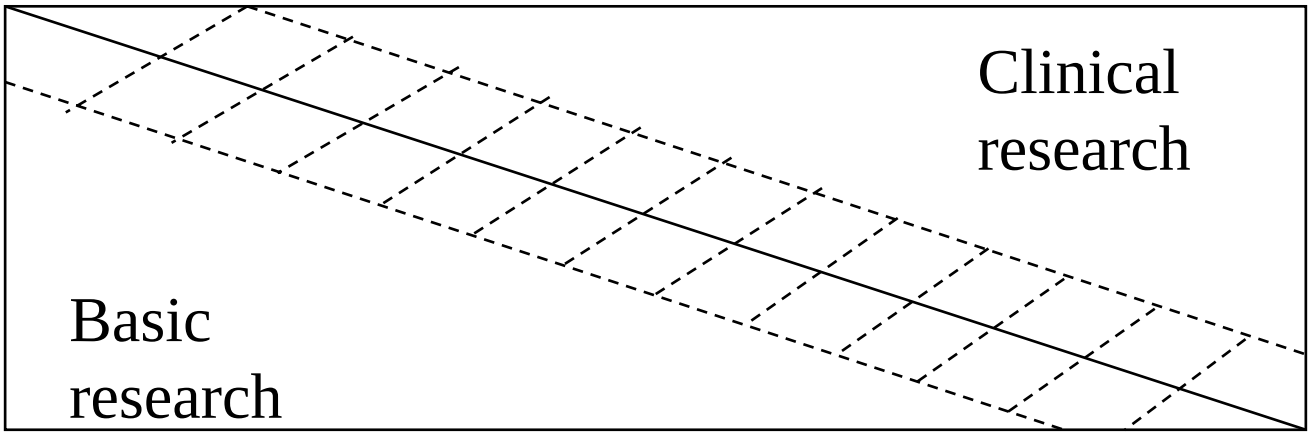
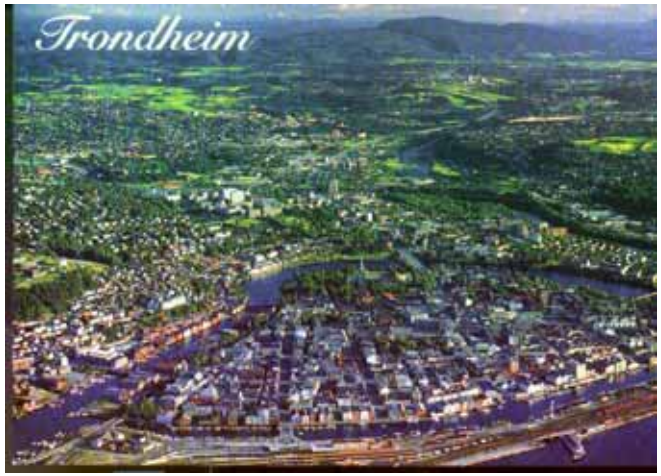
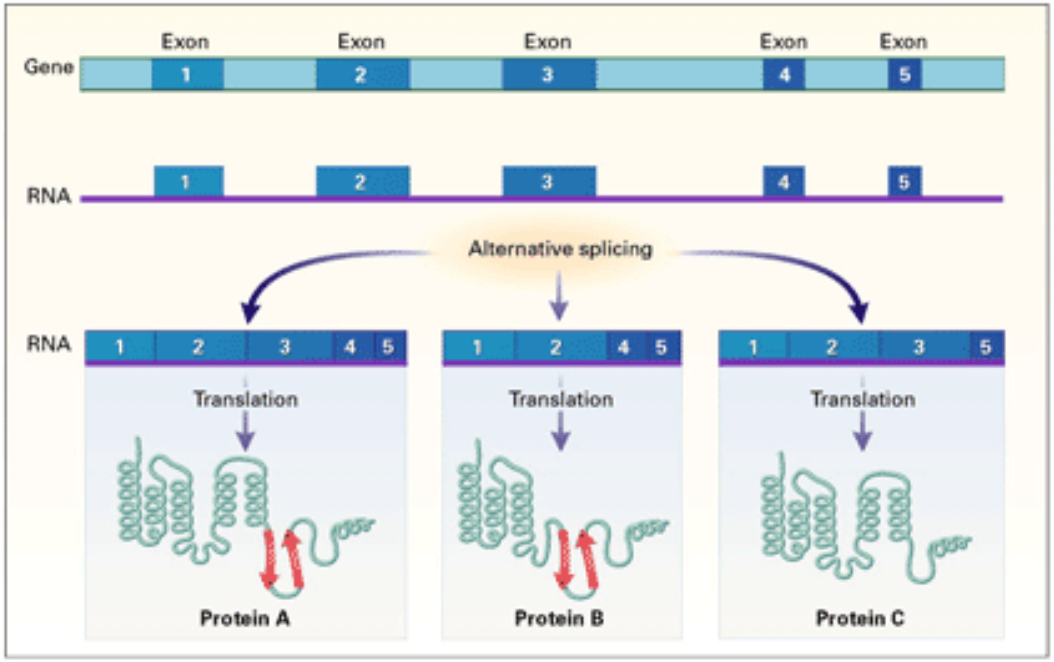
Translational research



Translational research is all about working together

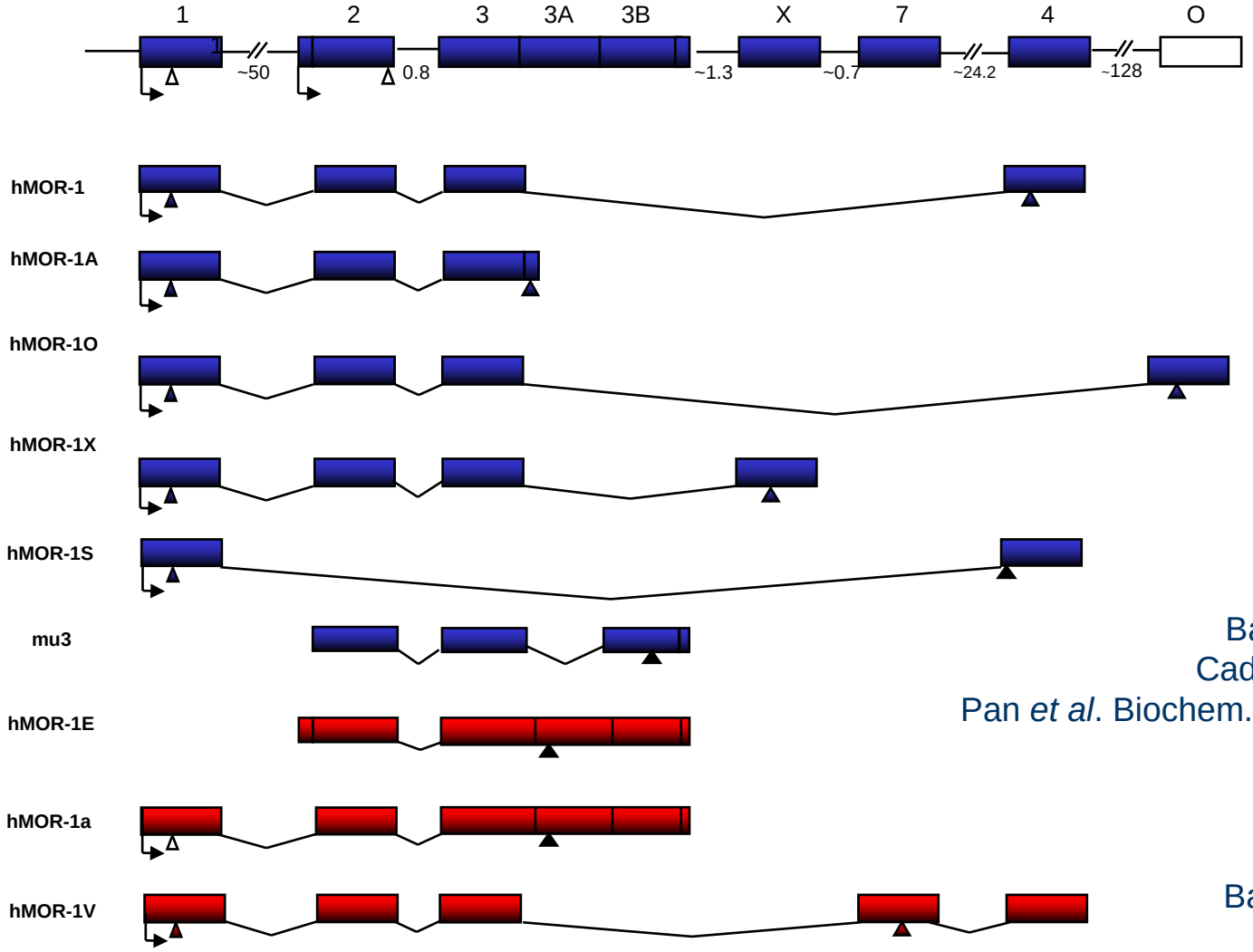


Genetic variability 1 - Splicing



NEJM Guttmacher 2002

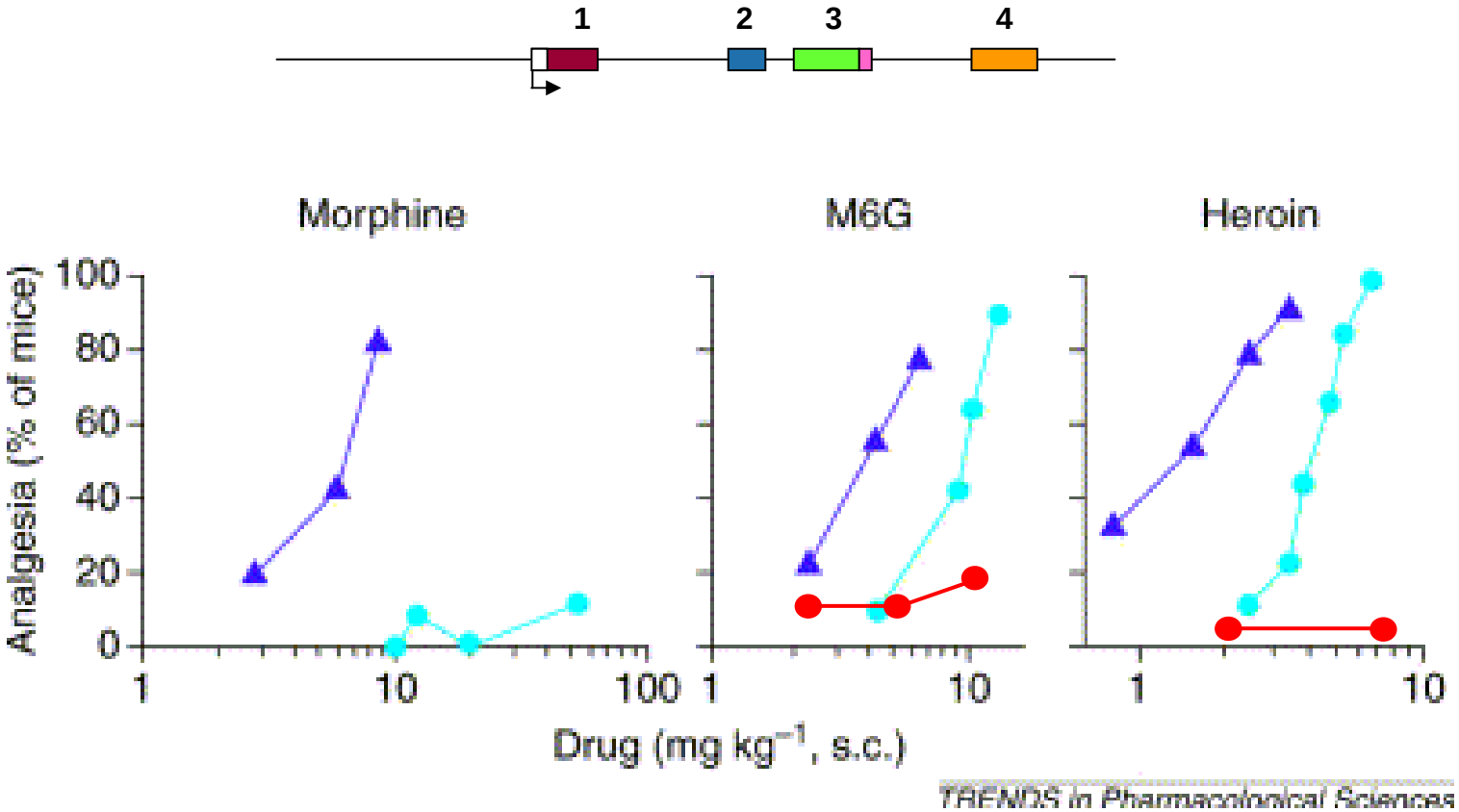
Splice variants mu-opioid receptor in human



Bare *et al*. FEBS Lett. 1994
Cadet *et al.* J. Immunol. 2003
Pan *et al*. Biochem. Biophys Res. Com. 2003

Baar *et al*. In manuscript

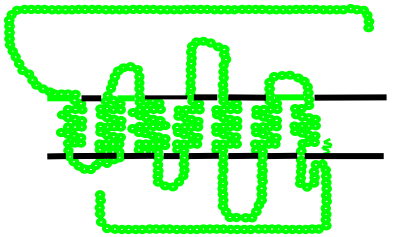
Oprm exon 2 “knockout” mus

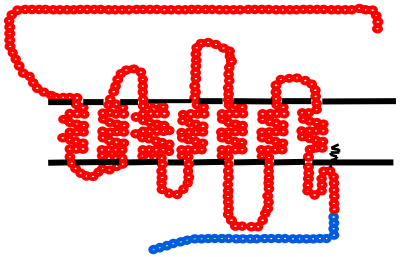


Pasternak GW, Trends Pharmacol Sci. 2001, **22**:67-70. Review.

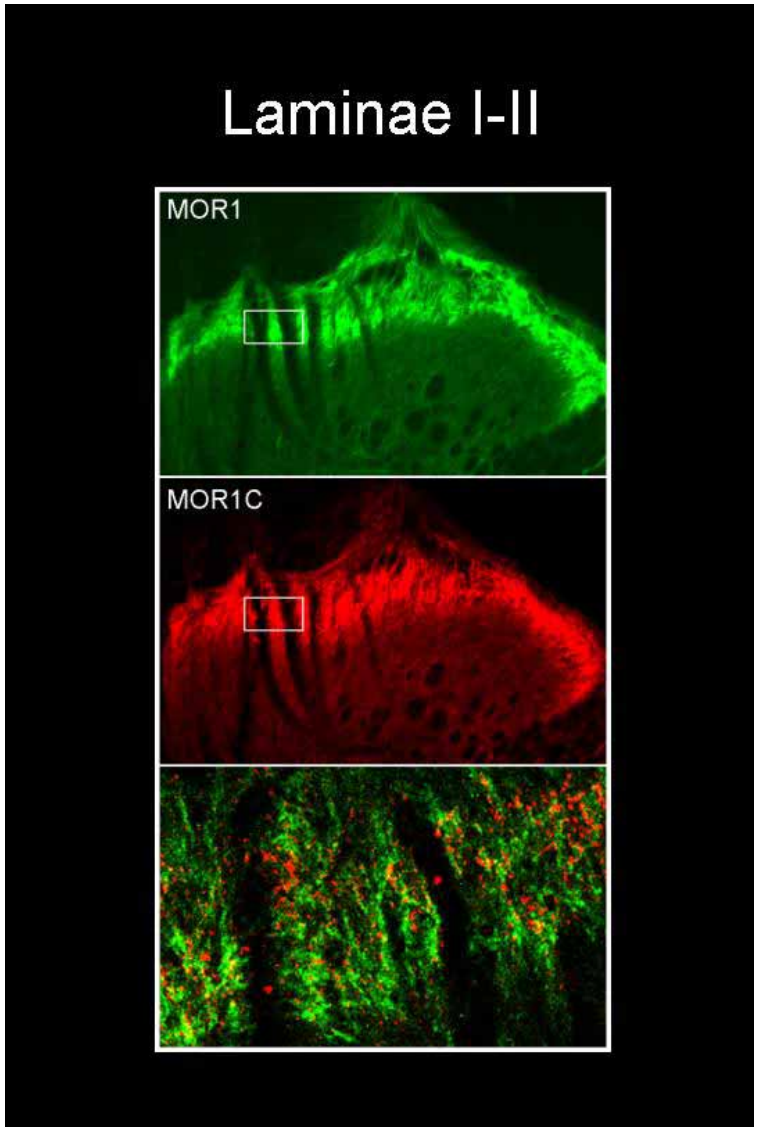
Schuller AGP et al., Nature Neurosci. 1999, **2**:151-156.

Splice variants MOR-1 og MOR-1C i dorsal horn





"Overlay"



Gavril W. Pasternak and co-workers,
Memorial Sloan-Kettering Cancer Center,
New York, US

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JPET 303:557–562, 2002

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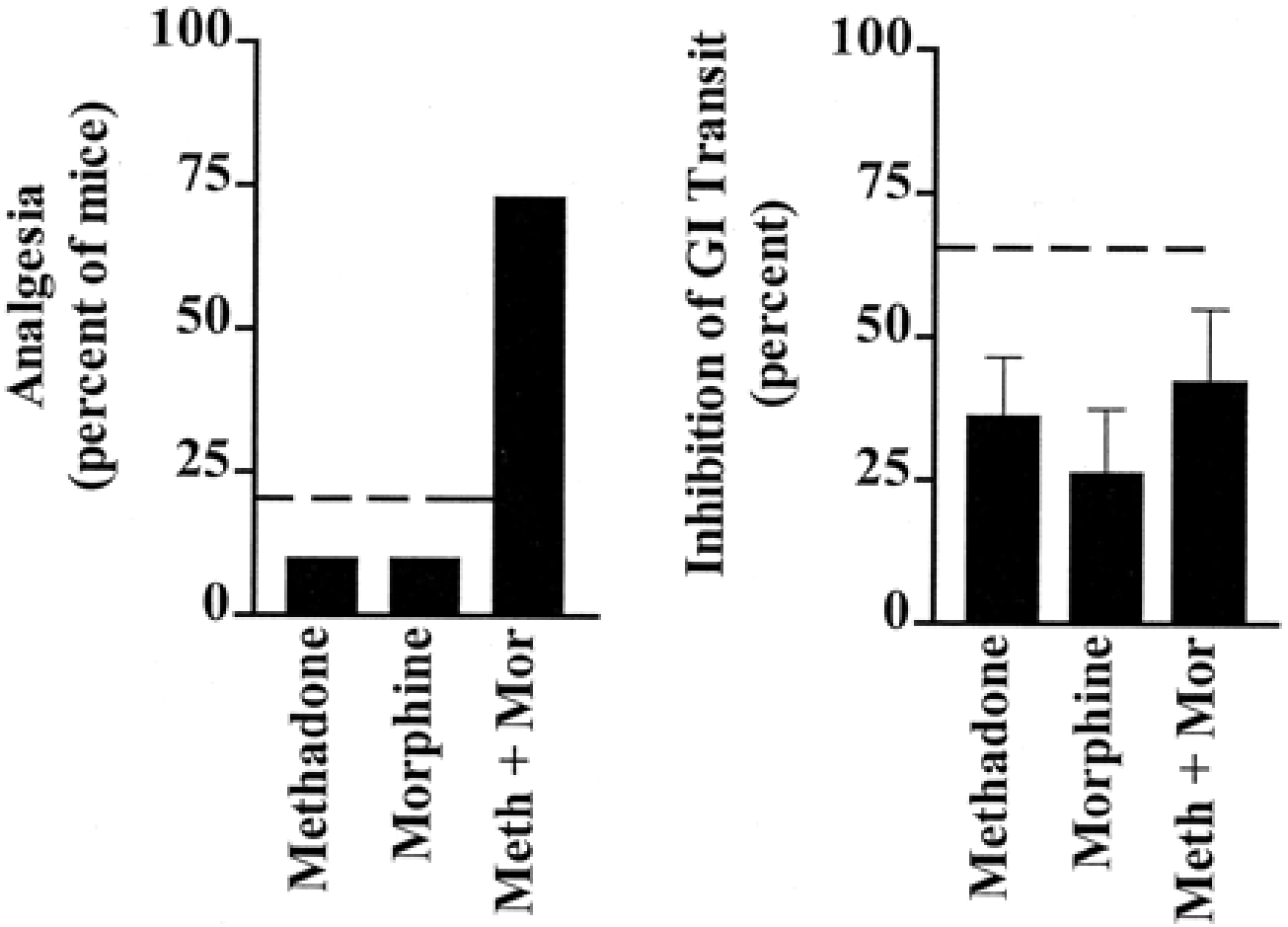
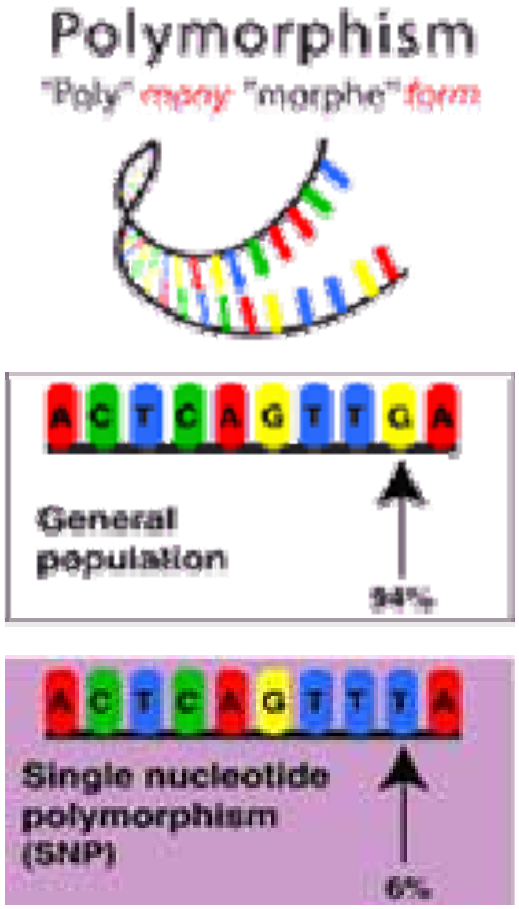
Synergy between μ Opioid Ligands: Evidence for Functional Interactions among μ Opioid Receptor Subtypes

ELIZABETH A. BOLAN, RONALD J. TALLARIDA, and GAVRIL W. PASTERNAK



Genetic variability 2:
Single Nucleotide Polymorphisms (SNPs)



Known SNP variability relevant to clinical opioid analgesia

- Opioid metabolizing enzymes (e.g. UGT2B7)
- Opioid receptors
- Drug transporters (MDR)
- Interacting systems (e.g. COMT)
- Joint effects of multiple genes and variations
- Opioid signaling (e.g. ß-arrestin, STAT6)

What do these studies tell us?

- Genetic variability is associated with opioid efficacy

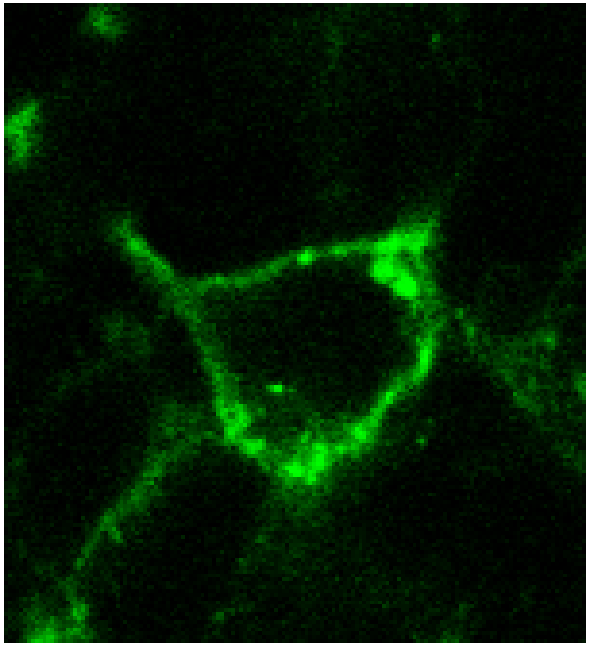
  and

  – Further clinical studies will confirm proposed associations

  – Further clinical studies will certainly establish new important genes

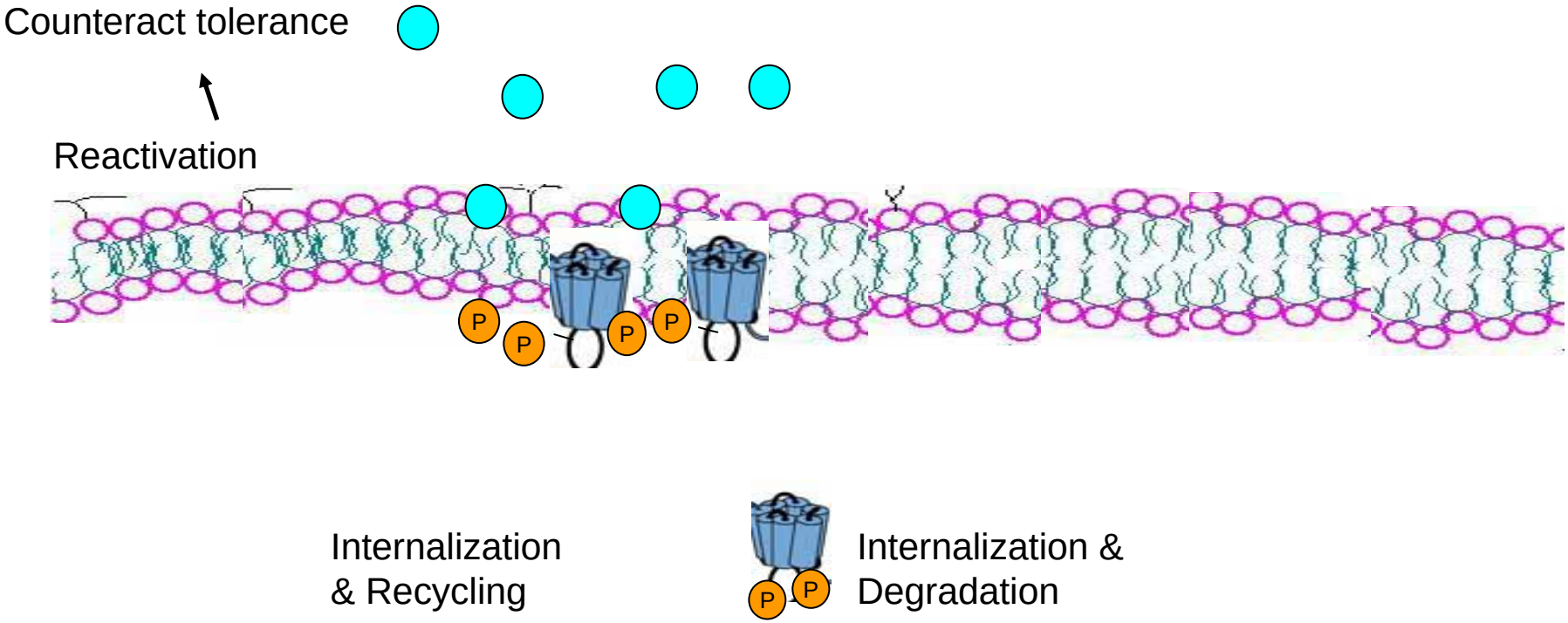
- But we don ´t know the functional consequences of genetic variability in genes related to opioid pharmacology

At this point the plan was to introduce the scheduled lecture “Internalization of opioids” by Cecilie Baar



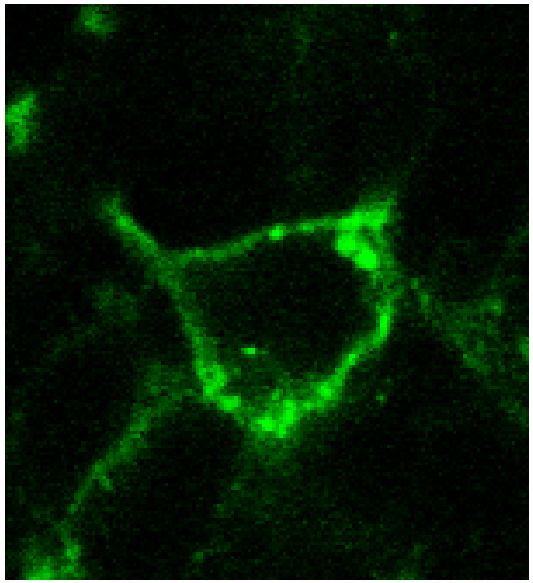
Why Cecilie is not telling this story herself to day

Localization of receptor after stimulation



Artwork by C Baar

Localization of receptor after stimulation



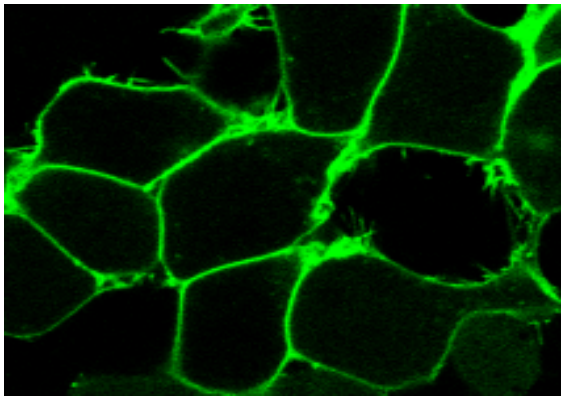
DAMGO binding to opioid receptors shown by confocal microscopy

Internalization:	
Ethorphine	+++
Morphine	-
Buphrenorphine	-
Fentanyl	+
Methadone	+

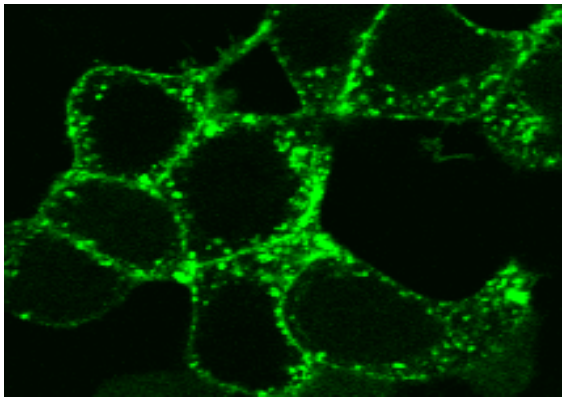
Kraus et al. J Biol Chem 2001

Difference between different opioids - difference between different receptor variants?

Functional characterization of opioid receptors by laser confocal microscopy



MEMBRANE ASSOSIATION

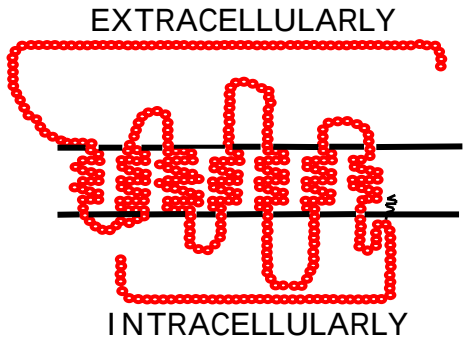


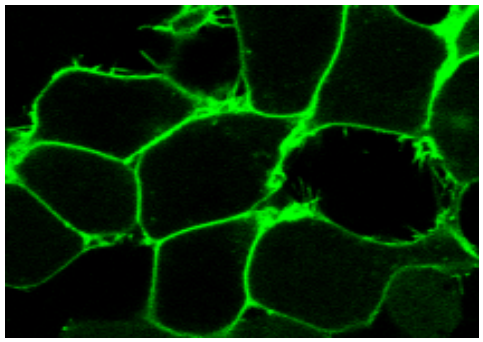
RECEPTOR ENDOCYTOSIS

- Transient and stable expression of fluorescence-tagged (GFP, CFP, YFP) μ opioid receptor subtypes in mammalian cells
- It is possible to produce cell lines with exactly the kind of genetic variability wanted
- Assessment of opioid-induced internalization and recycling

Functional characterization of opioid receptors by laser confocal microscopy: where are they localized?

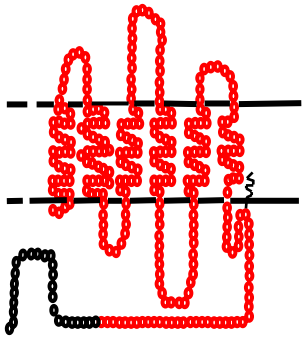
hMOR-1

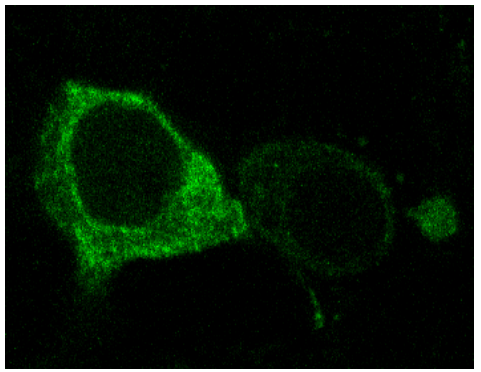




Membrane associated

hMOR-1AΔ



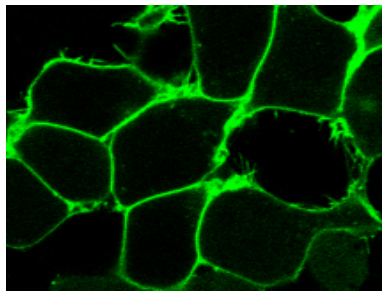
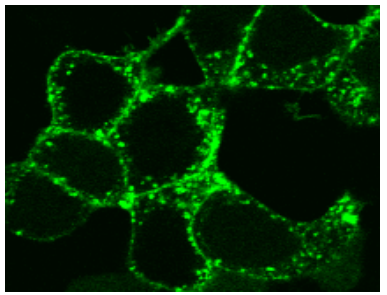
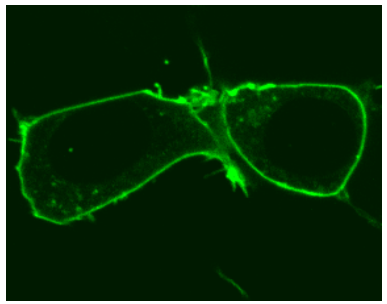
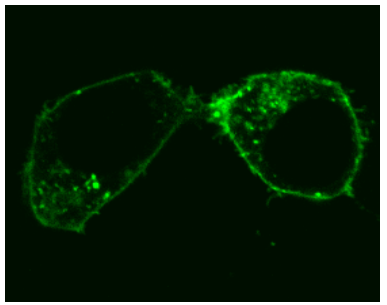
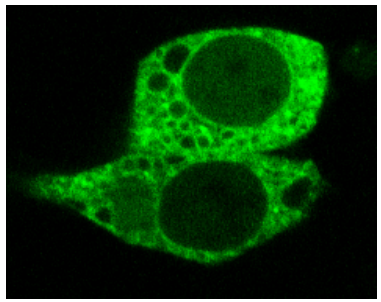
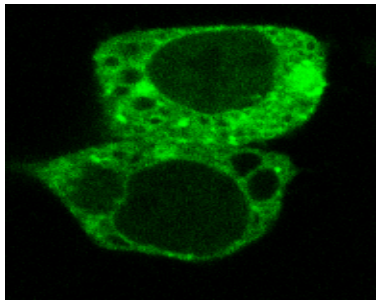


No accumulation in cell membranes, but spread all through the cytoplasm.

- Transient and stable expression of fluorescence-tagged (GFP, CFP, YFP) μ opioid receptor subtypes in mammalian cells

Functional characterization of opioid receptors by laser confocal microscopy: what happens upon ligand binding?

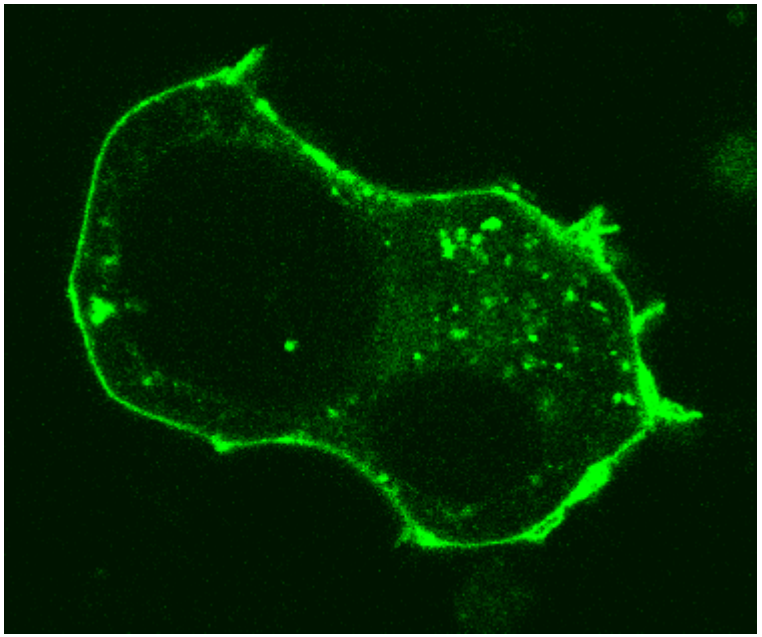
DAMGO, 1µM

	t = 0 min	t = 30 min	
hMOR-1			Receptor internalization
hMOR-1A			Receptor internalization
hMOR-1AΔ			No effect

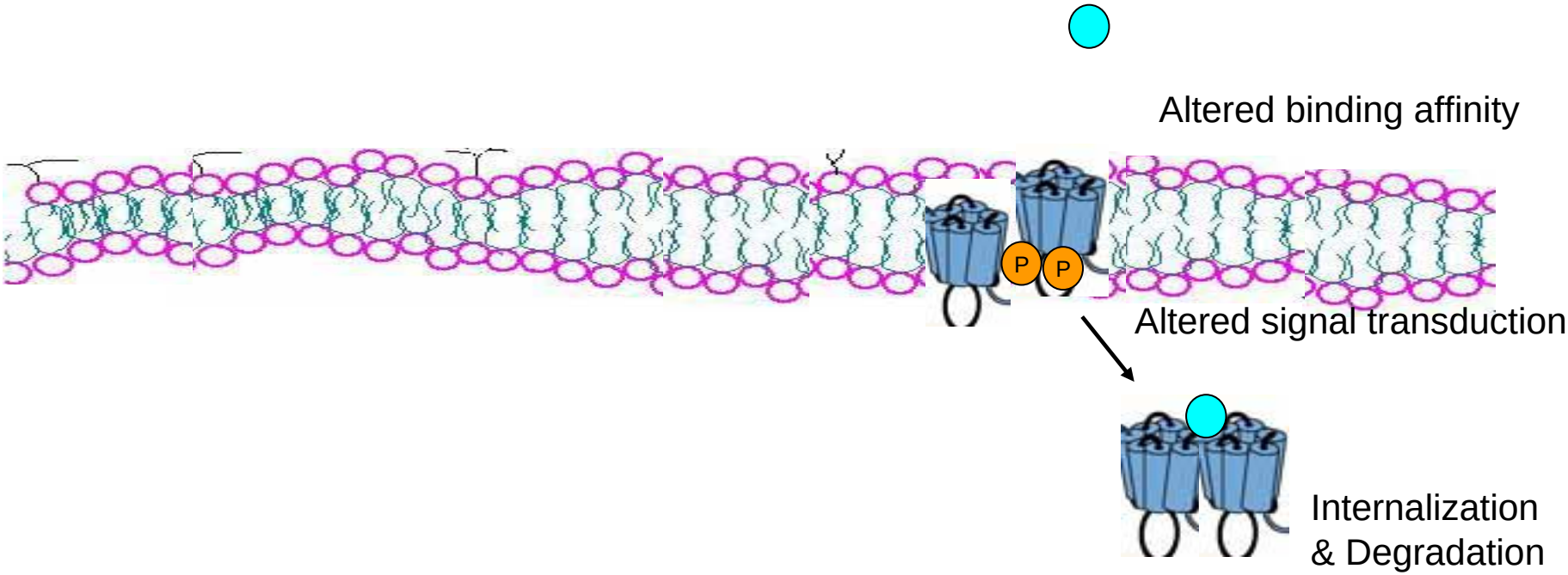
Latest news from the confocal:

”hMOR-1E”

A new splice variant, not published. Membrane associated but more intracellular granula than with hMOR-1.

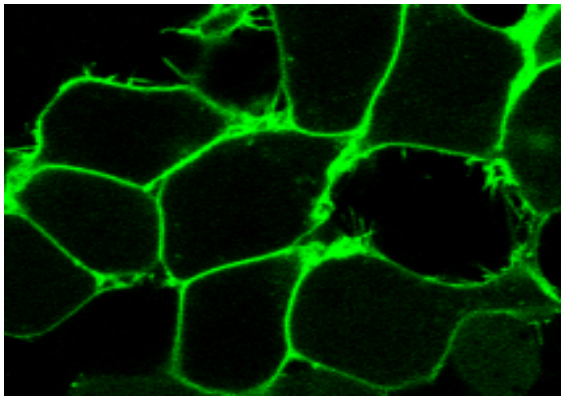


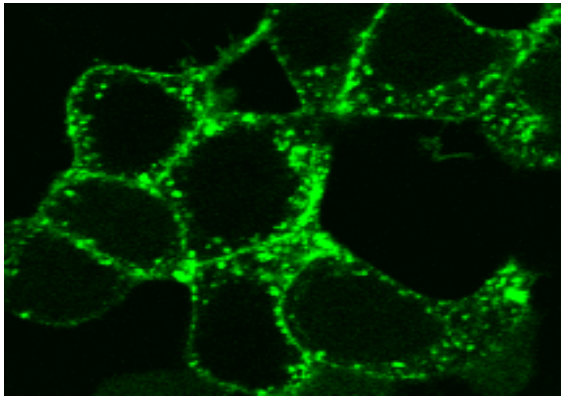
Localization of receptor after stimulation



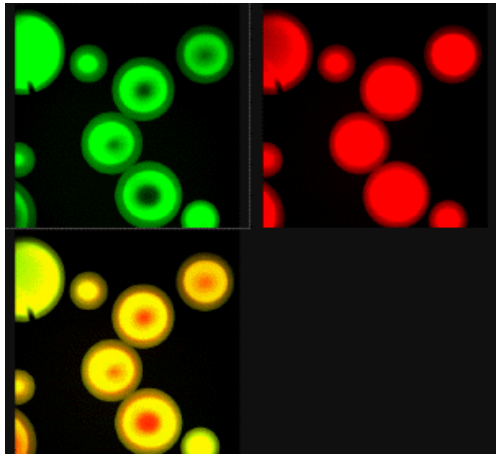
Artwork by C Baar

Functional characterization of opioid receptors by laser confocal microscopy

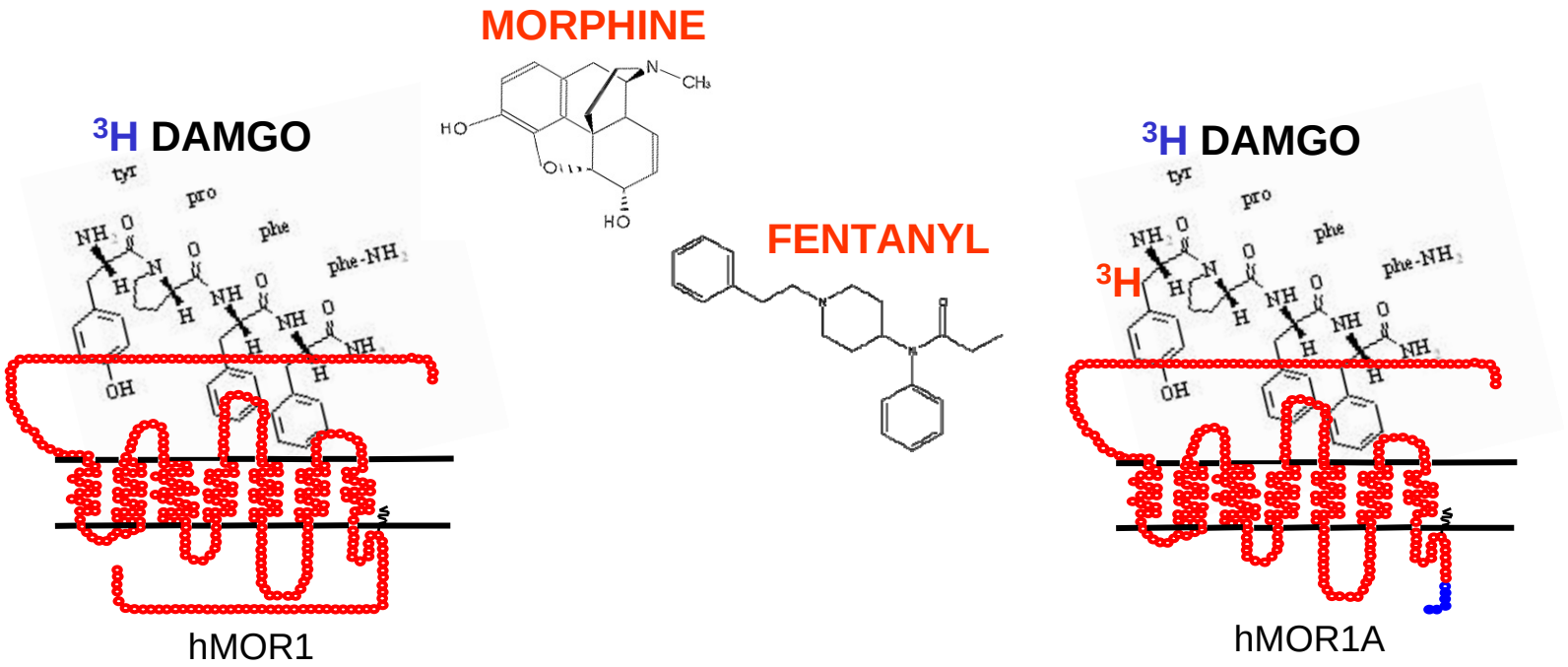




- Characterization of opioid receptor homo-/heterodimerization by FRET analysis
- Detects dimerization by closeness less than 50Å

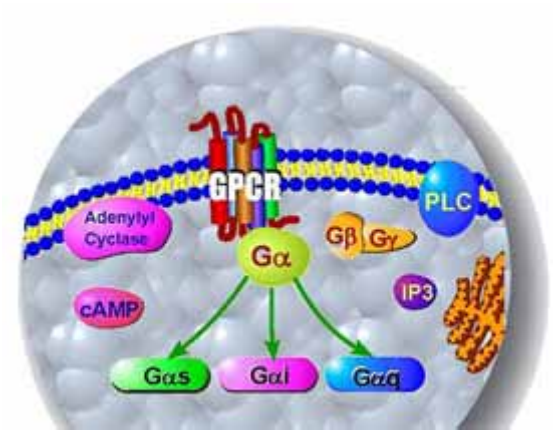


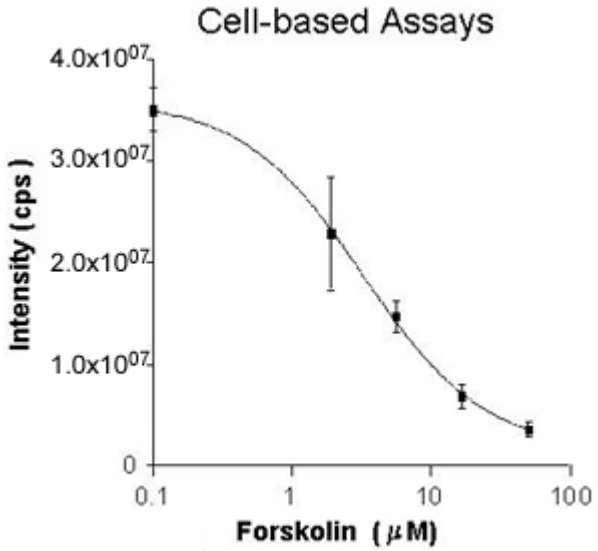
Functional characterization of μ opioid receptor variants: binding assays



- Competition assays against [^{3}H]-DAMGO in membrane fractions of cells expressing opioid receptors

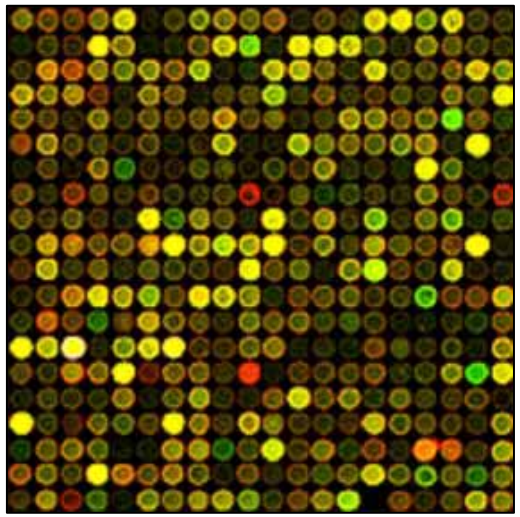
Functional characterization of μ opioid receptor variants: cAMP assays



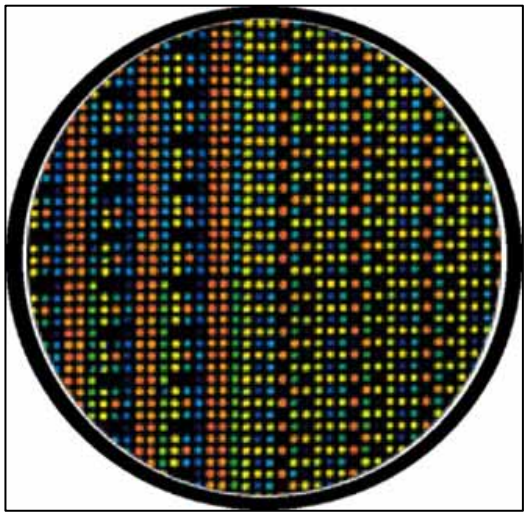


- Cell based screening of G-protein coupled opioid receptor activation. Measures intracellular levels of cAMP. Activation of μ opioid receptors inhibit adenylyl cyclase and lower the cAMP level.
- Comparisons between different receptor variants and different opioids.

Functional genomics - microarray



MICROARRAY
gene expression



MICROARRAY
SNP genotyping

- Changes in global gene expression caused by opioid exposure
- Different opioids – different patterns?
- Identification of “new” genes relevant to opioid responses

- Genome-wide SNP genotyping to assess interindividual variation in opioid responses
- Identify “core set” of SNP markers able to predict best opioid treatment for each individual

The nest 5 years should give

- More precise information about known genetic variability
  - Data from larger populations
  - Data from different ethnic populations
  - Other opioids than morphine
  - Other symptoms than pain
- Find new genes that influence opioid efficacy
- Find functional opioid mechanism influenced by genetic variability

And where to present this new information