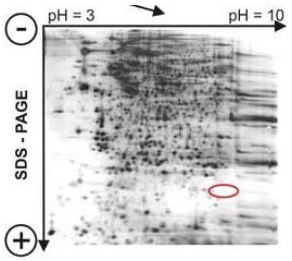
A third-generation method enables visualization of epigenetic marks in single cells

12.02.2013

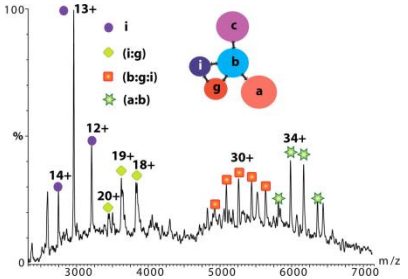
Kristin Fritsch

Methods currently use for standard protein detection

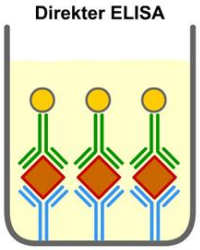
- 2D gel electrophoresis



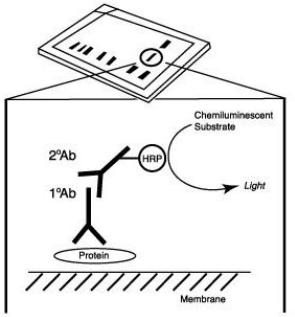
- Mass spectrometry



- ELISA



- Western blotting



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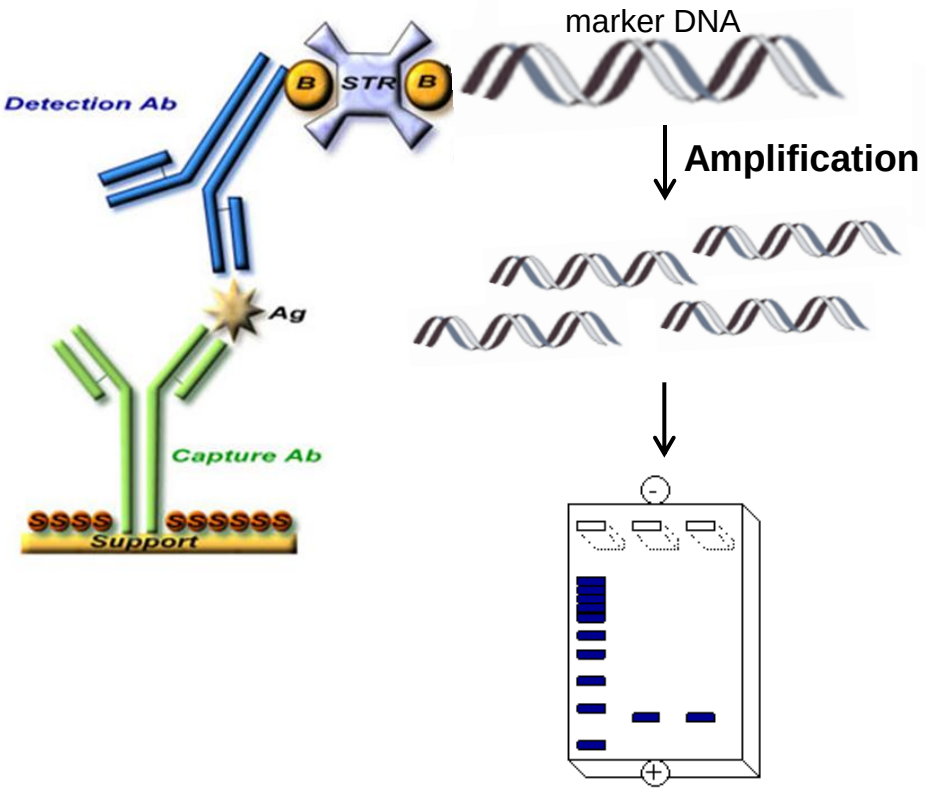
not sensitive enough to detect small amounts of protein

first-generation method – immuno PCR

- Detection of small amounts of protein *(T. Sano, Science 1992)*



- capture of antigen (direct on the plate or indirect by a capture molecule)
- recognition of the antigen by a detection antibody
- reporter DNA was bound using streptavidin
- reporter DNA was amplified using PCR
- gel electrophoresis of amplified DNA

modified from http://www.i-detect.eu/en_produ.php

first-generation method – immuno PCR

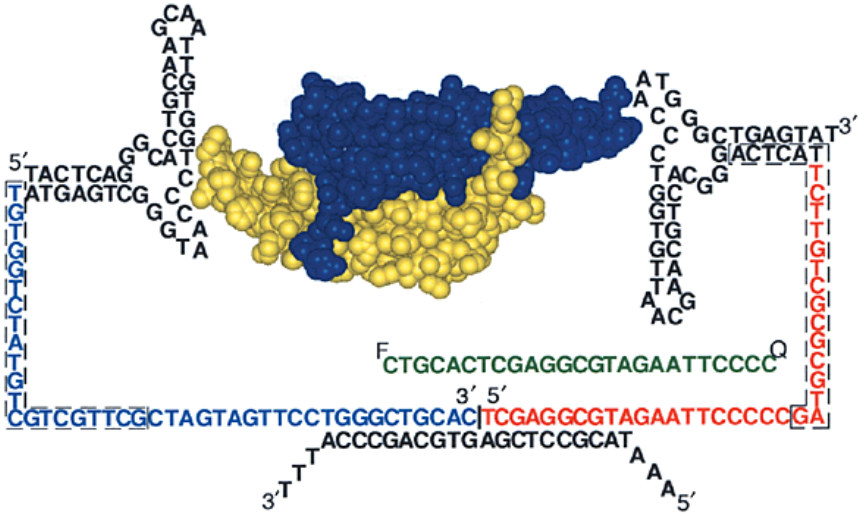
- avoidance of non-specific binding
- can performed under «real-time PCR» conditions
- increase in protein detection sensitivity by approximately 1000-fold
- usability for medically relevant antigens, e.g. Hepatitis B surface antigen
- detection of antigens from unpurified samples, e.g. serum

↓

The immuno-PCR provides an ultrasensitive technology which combines the molecular specificity of antibodies with the sensitivity of the PCR

second-generation method - proximity ligation assay (PLA)

- DNA-based protein detection assay *(S. Fredriksson et al., Nature Biotechnology 2002)*
- *in vitro* analysis of proteins and other macromolecules

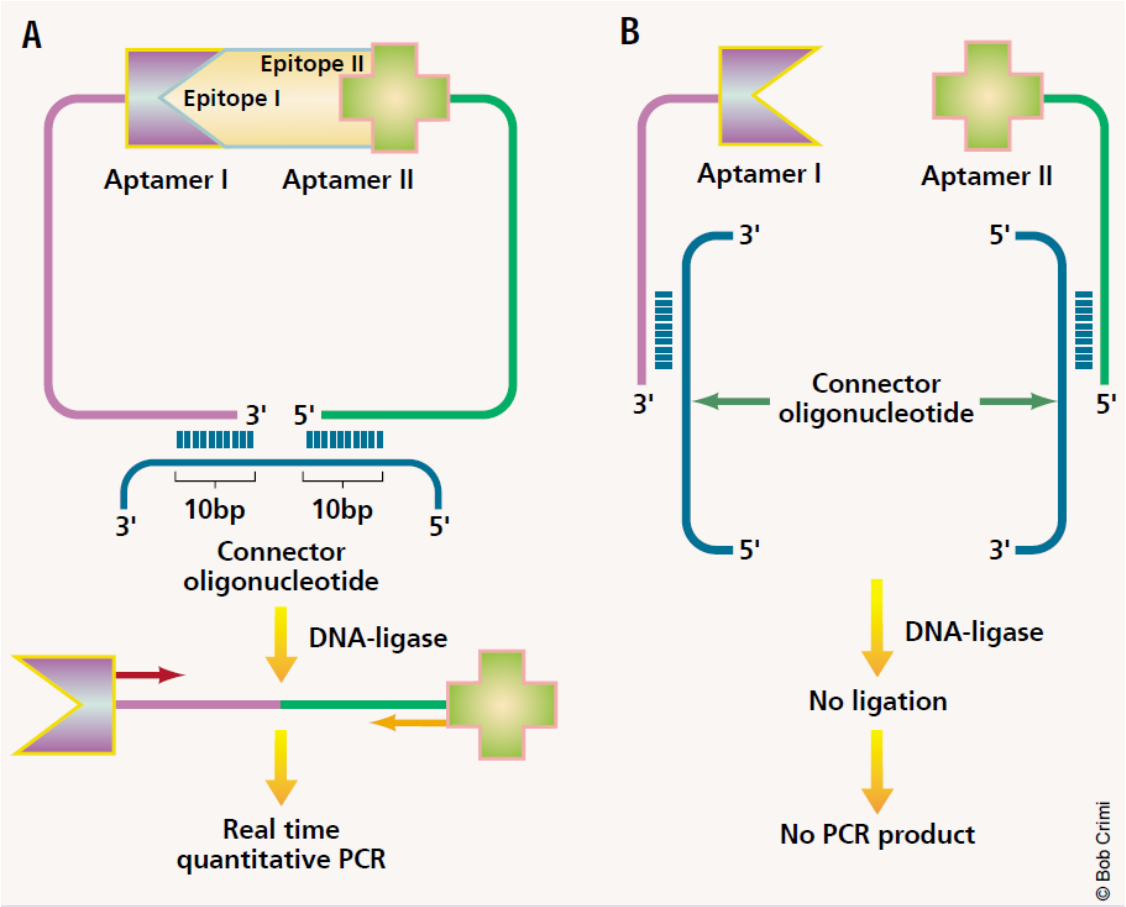


- pair of DNA aptamers binds to target protein
- each aptamer with different DNA- sequence extension
- binding of aptamer pair brings ends of oligonucleotide extensions into proximity
- connecter oligonucleotide hybridize to both ends
- amplification of PCR template

S. Fredriksson et al., Nature Biotechnology 2002

second-generation method - proximity ligation assay (PLA)

- *in vitro* analysis of proteins and other macromolecules



Michael Famulok, Nature Biotechnology 2002

second-generation method - proximity ligation assay (PLA)

- assay can be performed in a homogenous format
- suitable for automation
- potential for application in clinical laboratories
- aptamers can be replaced by antibodies
- difficult to adapt method to small organic molecules or small peptides

↓

method allows the detection and quantification of minute amounts of a specific protein but can not be used for quantifying small molecules

In situ proximity ligation assay (ISH-PLA)

- localization of protein-protein interactions at single molecule resolution

(O. Söderberg et al., Nature Methods 2006)

In situ proximity ligation assay (ISH-PLA)

- localization of protein-protein interactions at single molecule resolution

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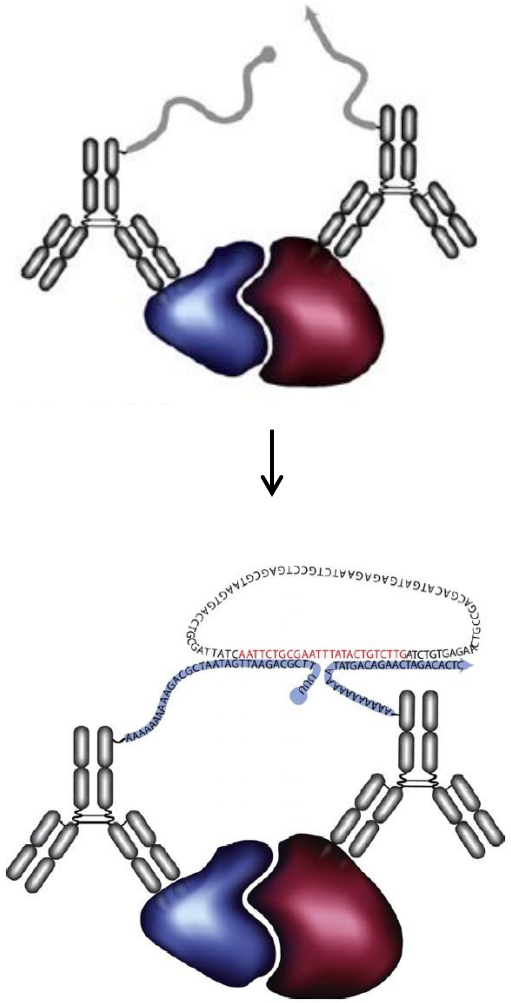
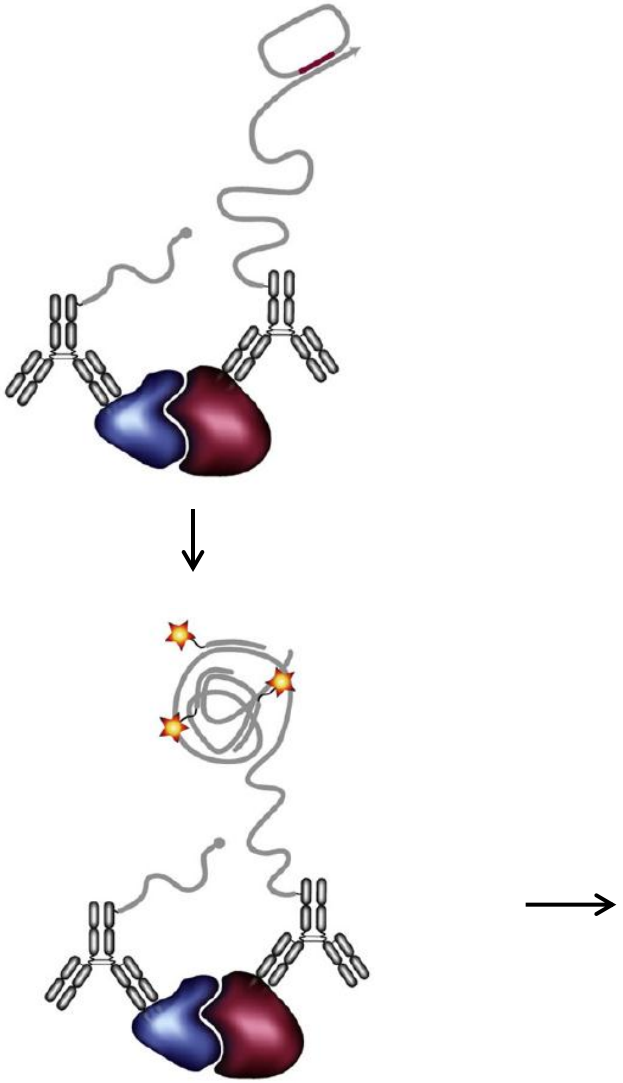


- Proximity probe binding
- circularization and ligation of connector oligonucleotides

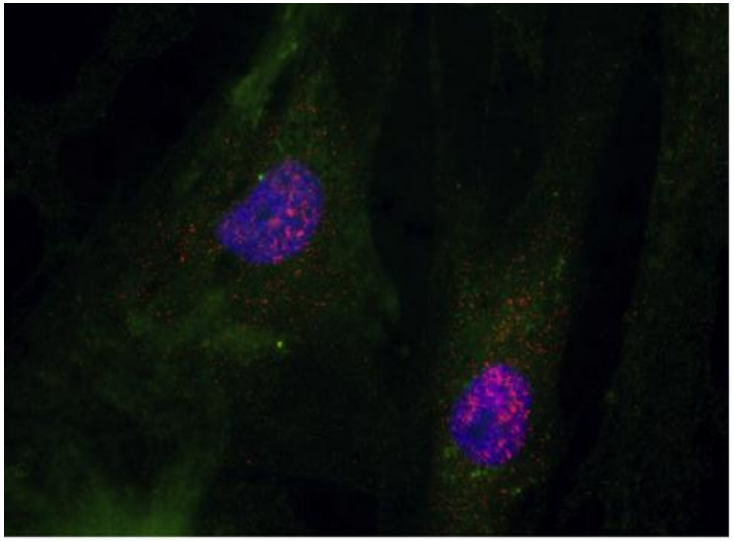
(O. Söderberg et al., Methods 2008)

In situ proximity ligation assay (ISH-PLA)

- localization of protein-protein interactions at single molecule resolution



- Rolling circle amplification
- Detection of rolling circle products



c-Myc/Max heterodimers in cultured human fibroblasts

(O. Söderberg et al., Methods 2008)

In situ proximity ligation assay (ISH-PLA)

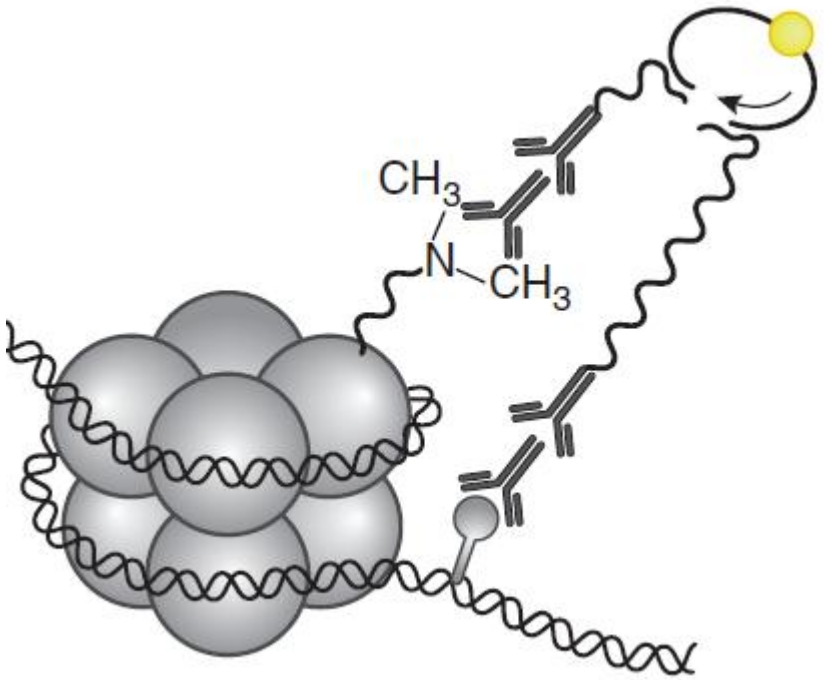
- analyses of interactions among any proteins for which antibodies are available
- assay can be performed in all samples of cells and tissues
- useful to monitor the effect of pharmaceutical treatment
- *in situ* PLA may find important uses in medical research, drug development, and clinical diagnostics

allows highly specific imaging of proteins and protein complexes in tissue samples

third-generation method - ISH-PLA

- detection of Histone modifications at single genomic locus

(D. Gomez et al., Nature Methods 2013)



- biotinylated probe target the gene of interest
- Another probe target chromatin modification
- 2nd Antibody with PLA
- Rolling circle amplification
- Detection of rolling circle products

Andrew P Feinberg, Nature Methods 2013

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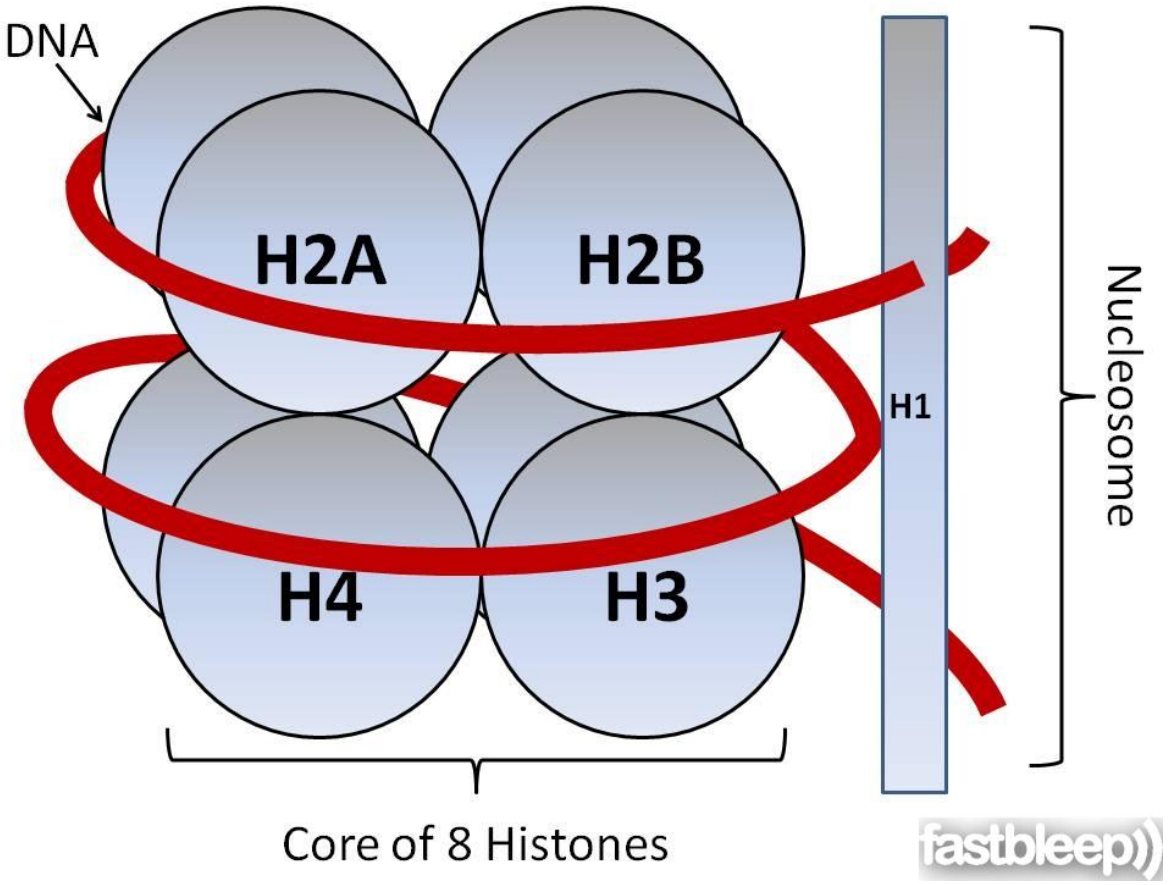
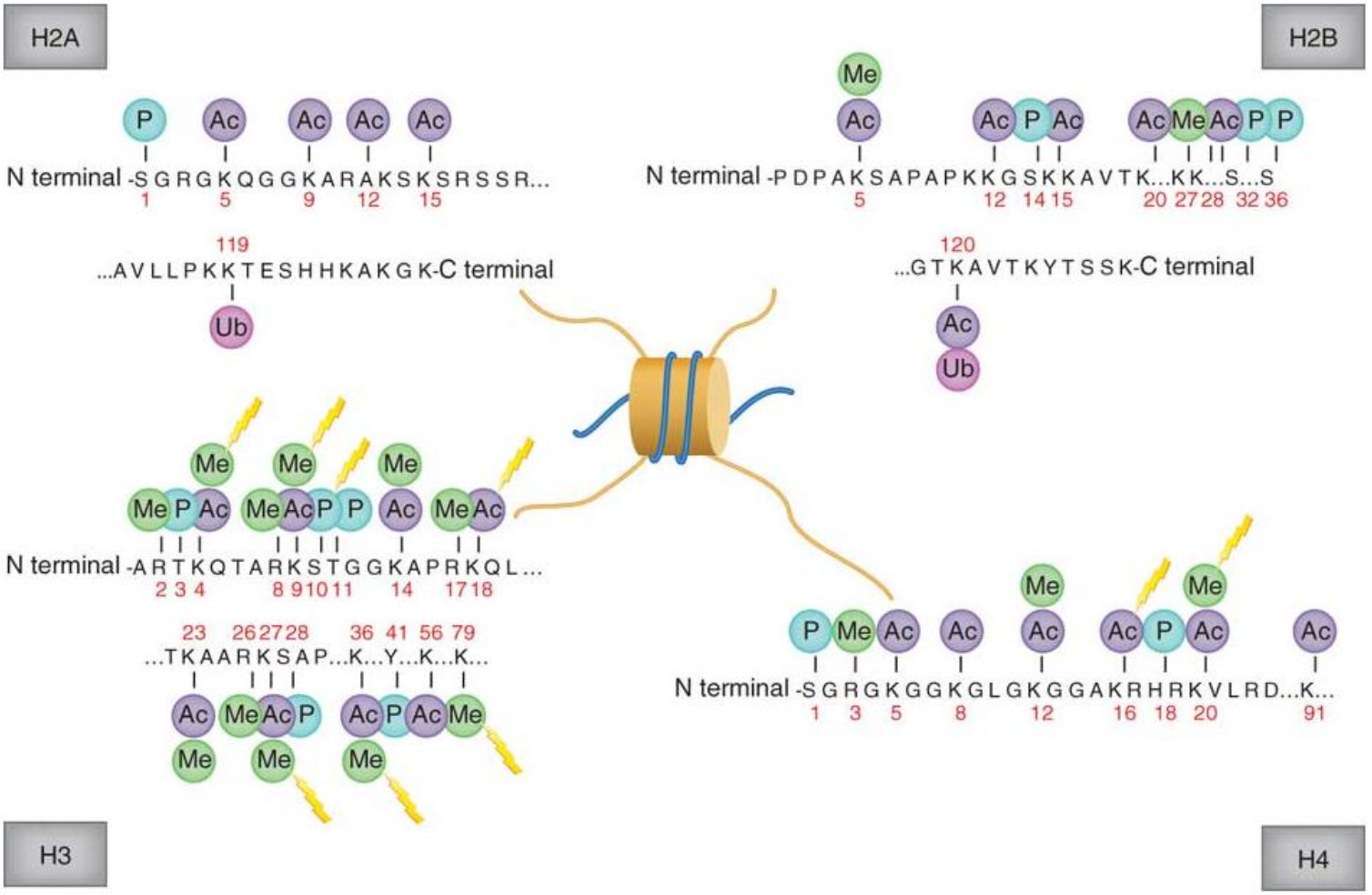
Detection of histone modifications at specific gene loci in single cells in histological sections

Delphine Gomez[1,2], Laura S Shankman[1,2], Anh T Nguyen[1] & Gary K Owens[1]

histone structure



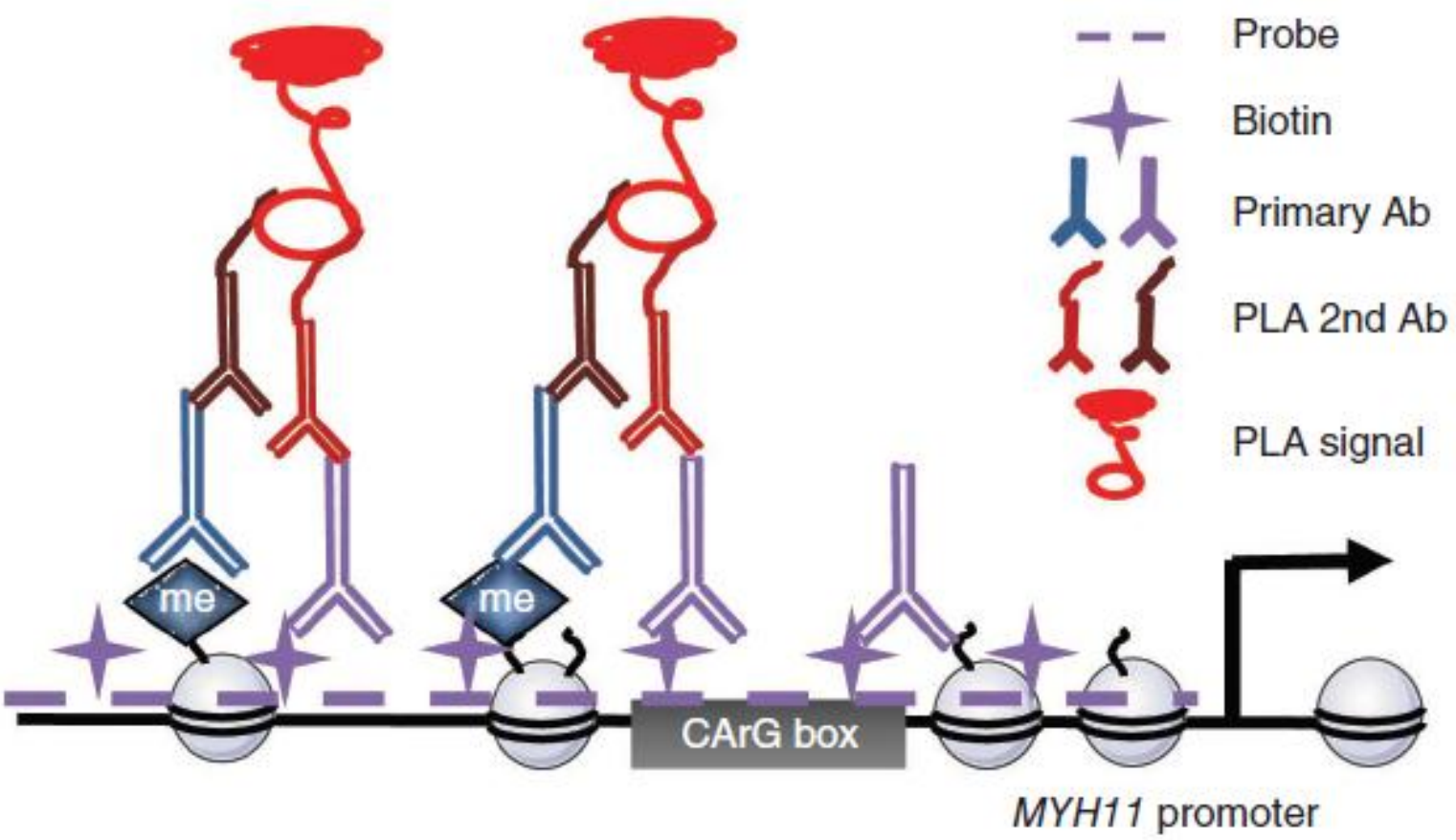
post-translational histone protein modifications that can influence epigenetic regulation of gene transcription



Manuel Rodríguez-Paredes & Manel Esteller, Nature Medicine 2011

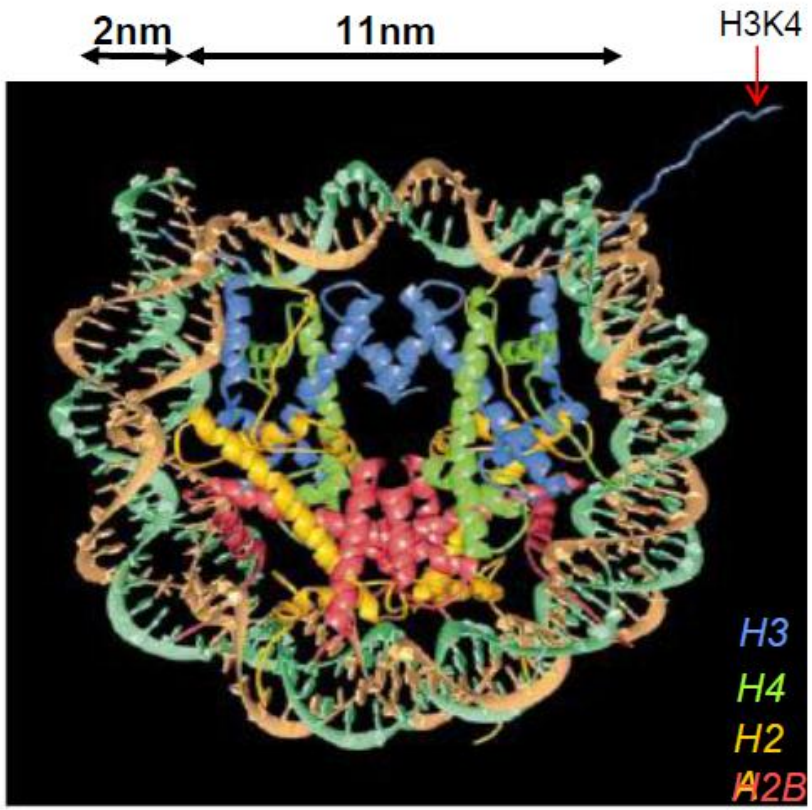
ISH-PLA detection of of H3K4me2 at MYH11 locus
In human coronary arteries
(highly relevant to atherosclerotic disease)

third generation method - ISH-PLA – detection of Histone modifications at single genomic locus



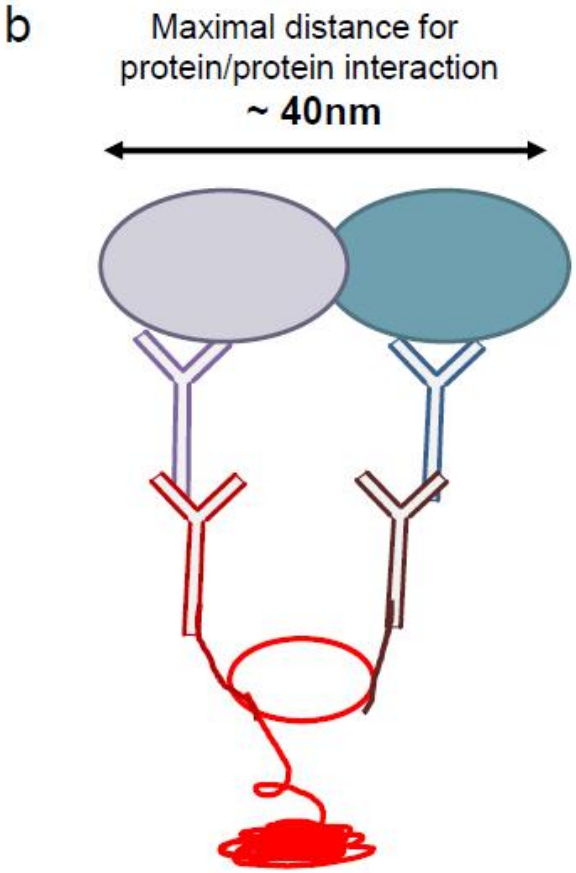
Compatibility between PLA and chromatin structure

a

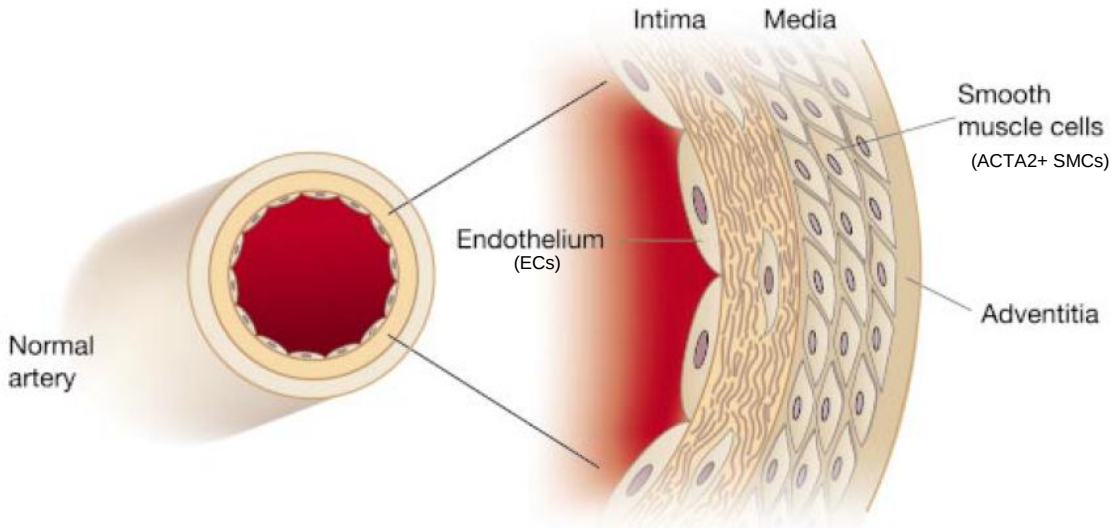


Estimated distance between two biotinylated ATPs within the DNA strand ~2 nm

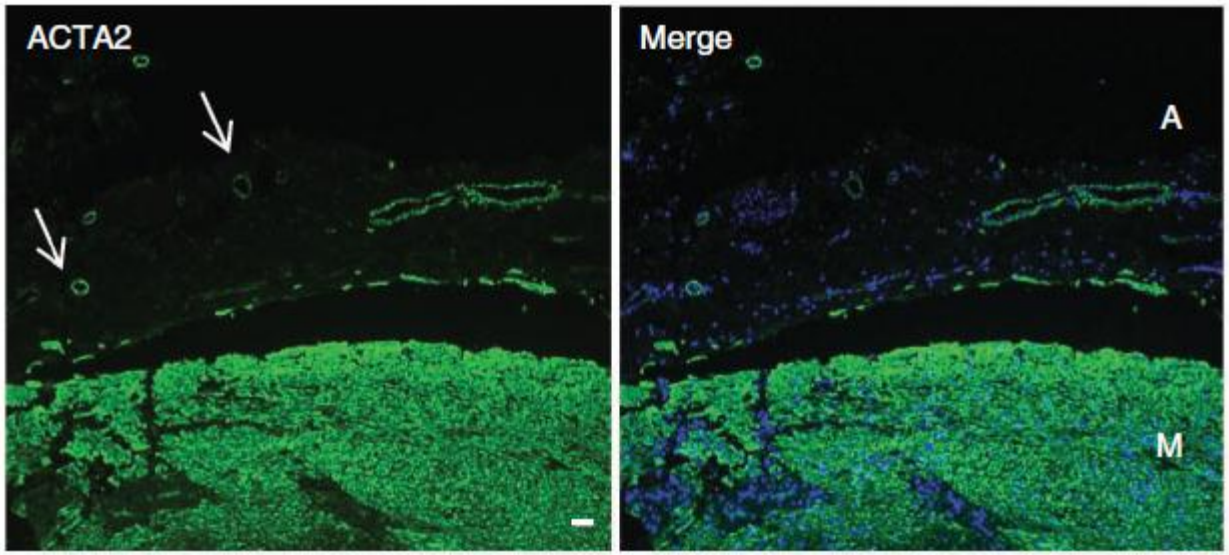
b



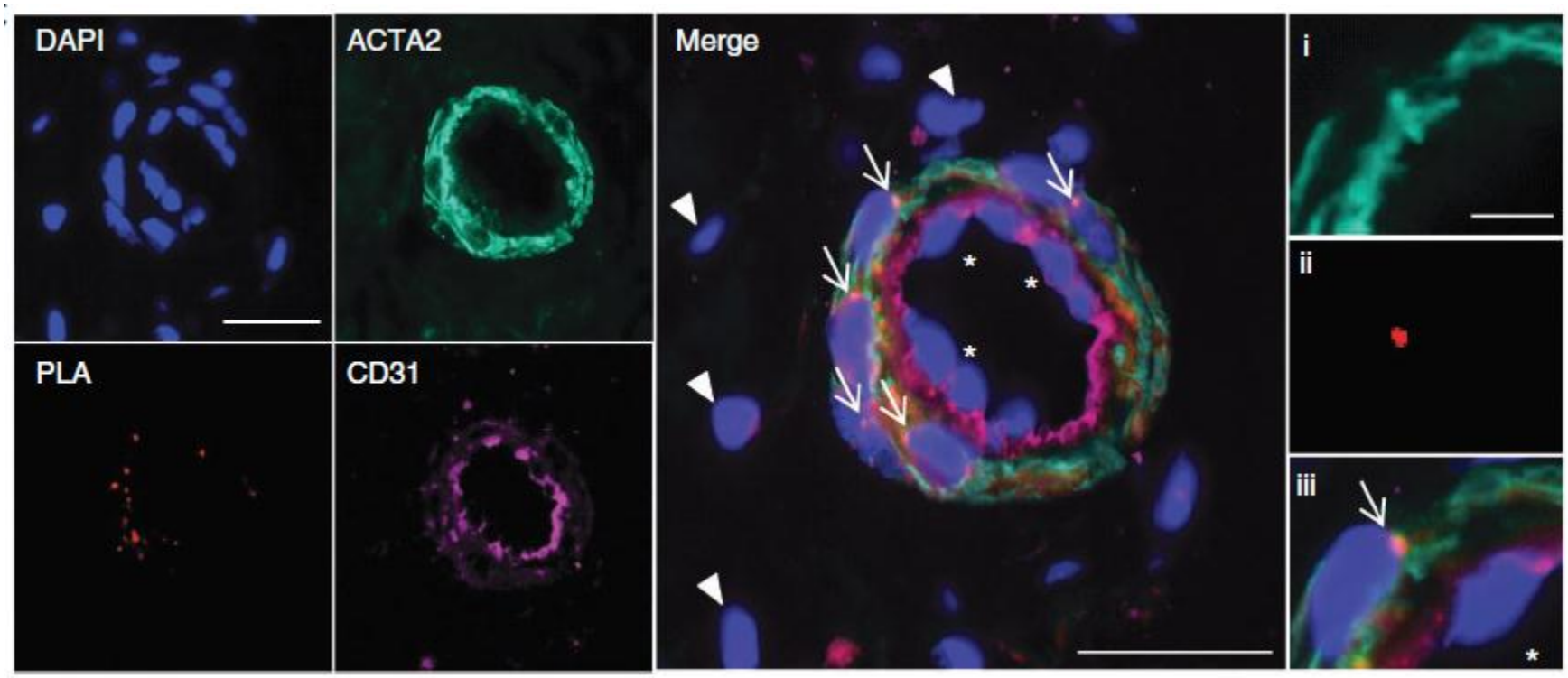
human coronary arteries



Peter Libby, Nature 2002

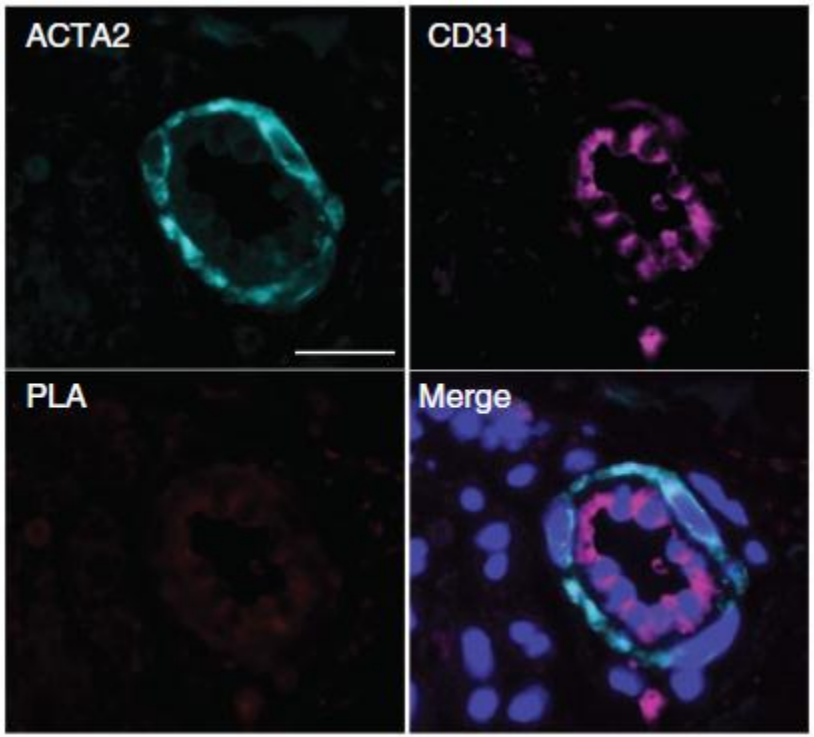


ISH-PLA detection in adventitial small arteries



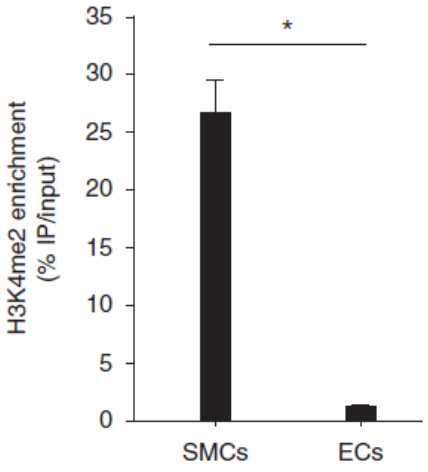
→ *MYH11* H3K4me2 PLA+ within ACTA2+ SMCs

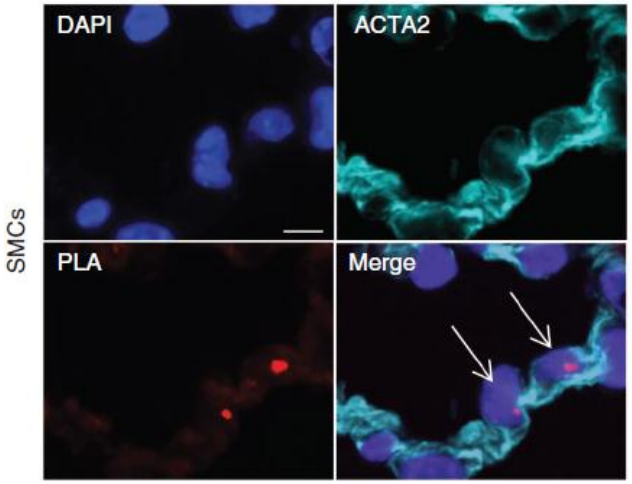
ISH-PLA negative control in an adventitial vessel of human carotid artery sections

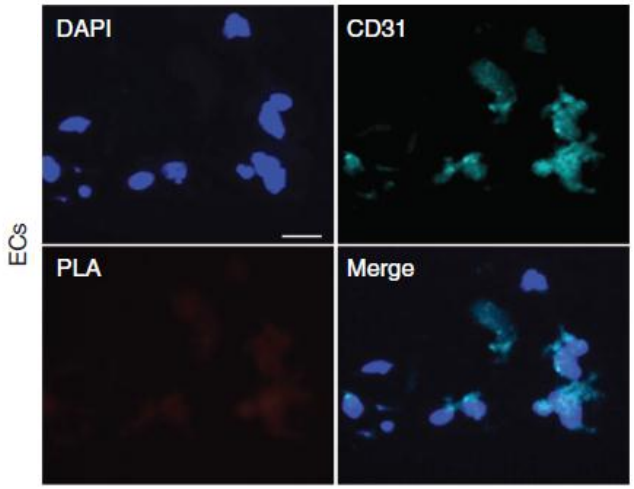


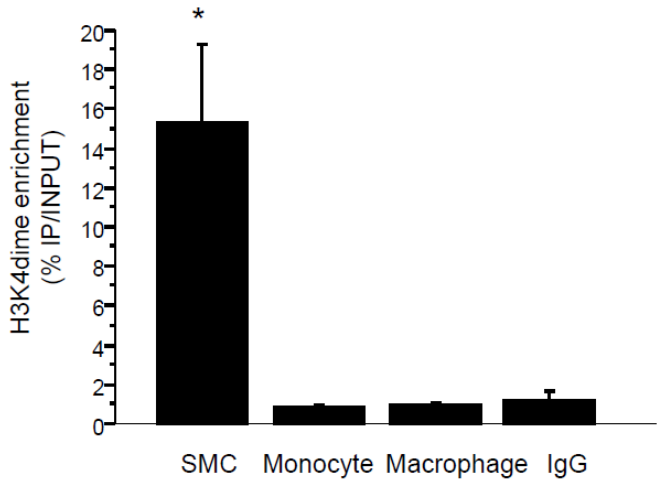
⟶ *MHY11* probe required for PLA amplification

comparision of ISH-PLA and CHIP assays





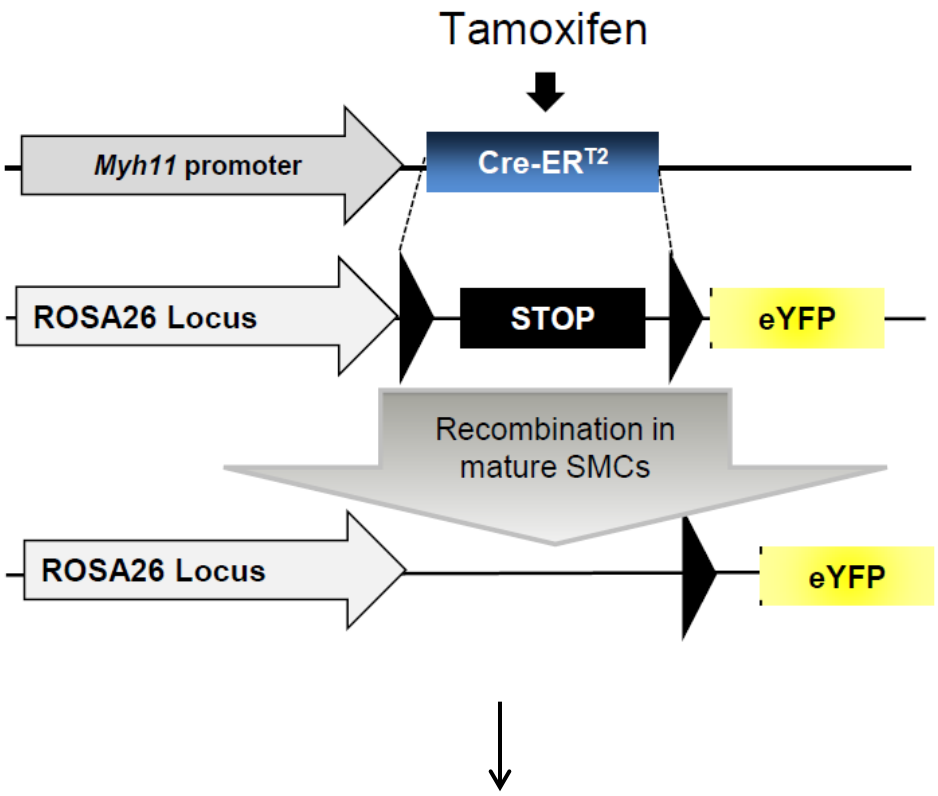




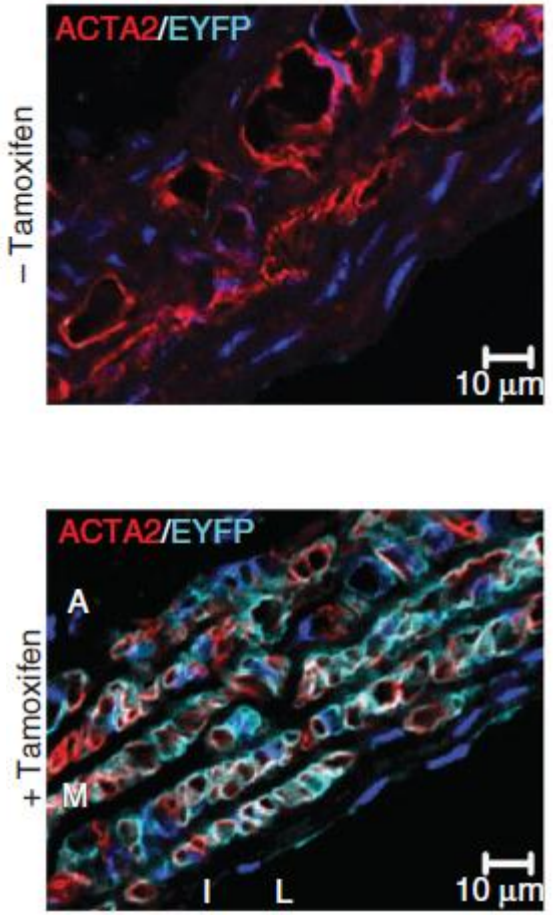
⟶ ChIP and ISH-PLA analyses showed H3K4me2 enrichment of MYH11 locus exclusively in SMCs

Assessment of eYFP expression in *Myh11 Cre*ERT2 ROSA26 STOP flox eYFP+/+ and eYFP–/– mice

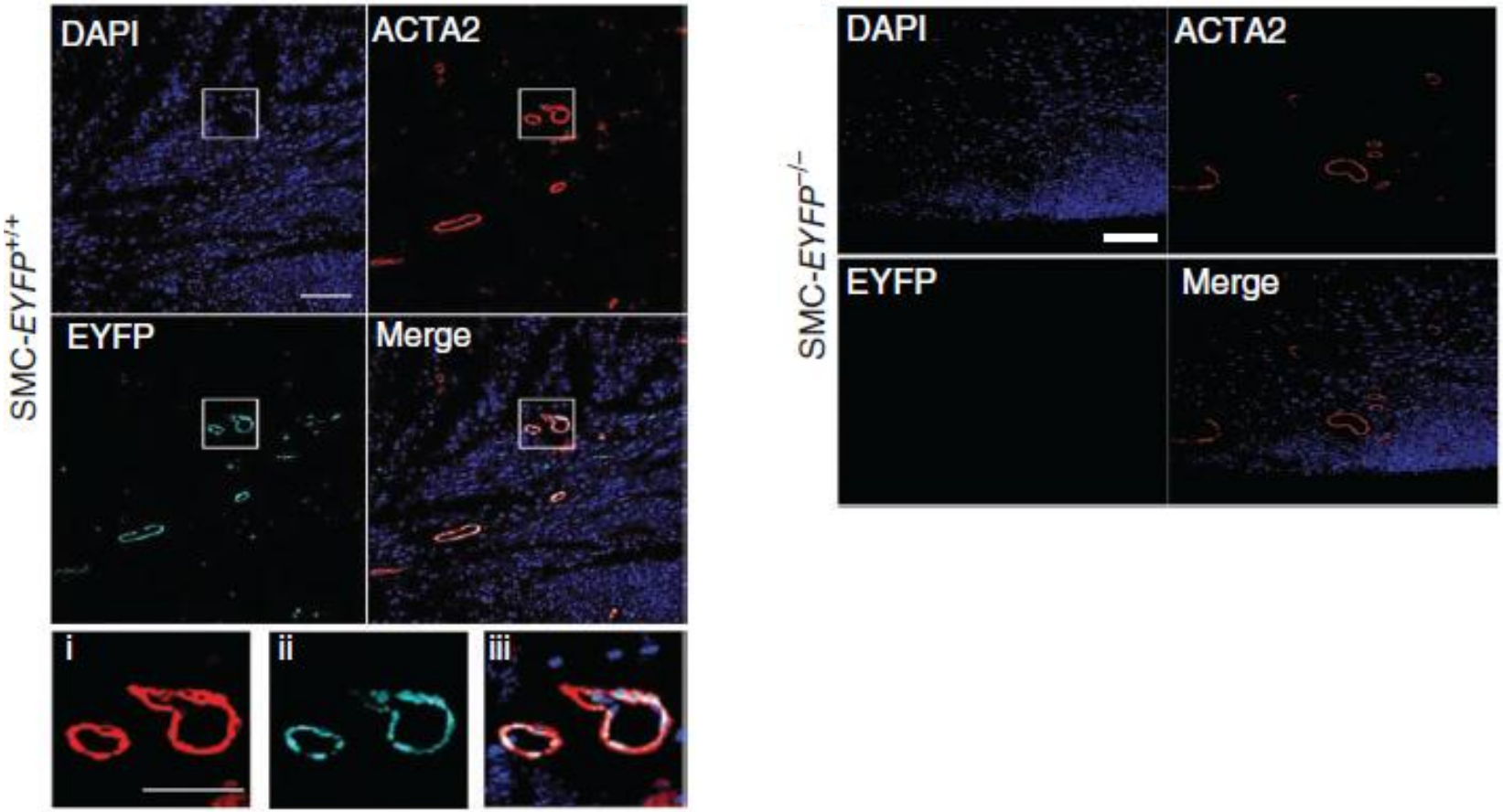
SMC lineage–tracing system



SMC-EYFP$^{+/+}$ mice

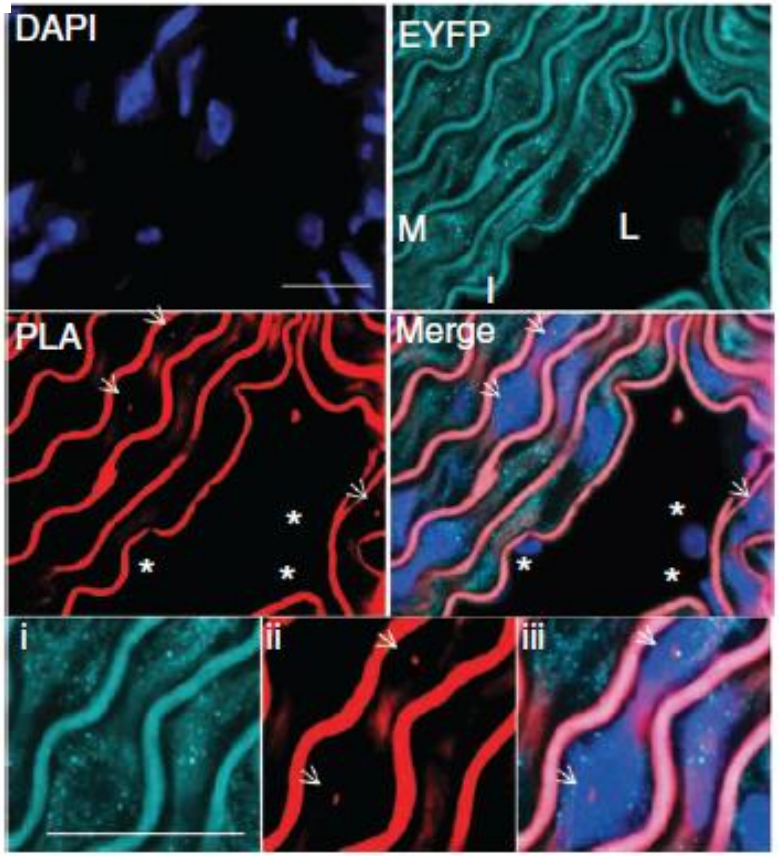


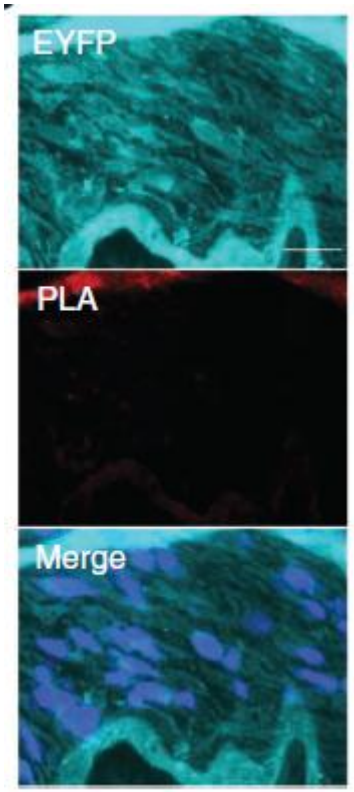
EYFP expression in heart tissue sections



⟶ high-efficiency EYFP expression exclusively in SMCs

ISH-PLA analysis of aortas from SMC-*EYFP*+/+ mice

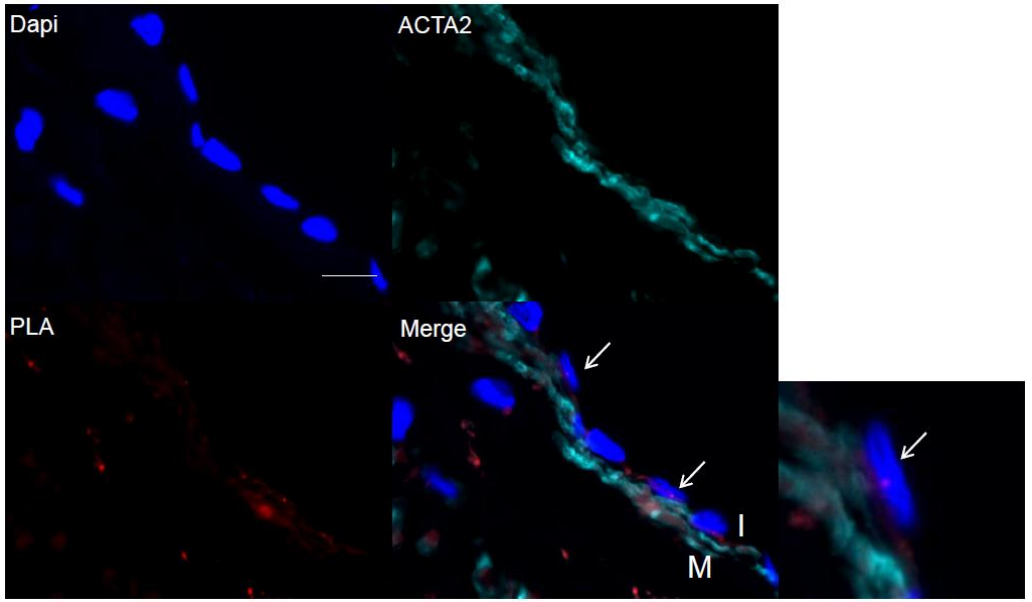




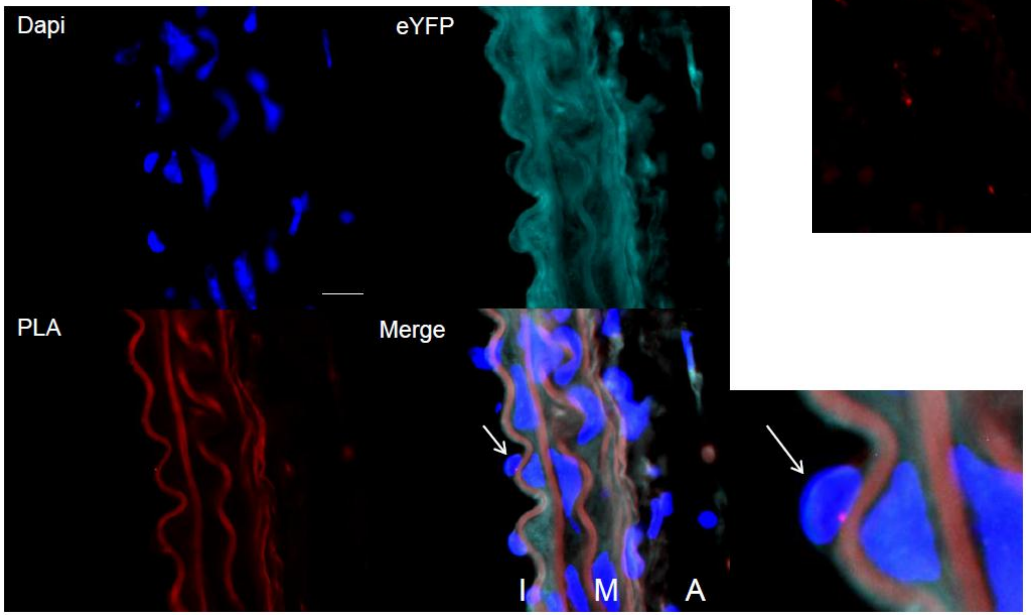
→ high-efficiency EYFP expression exclusively in SMCs

Cdh5 H3K4dime ISH-PLA assays

Human cartid arteries



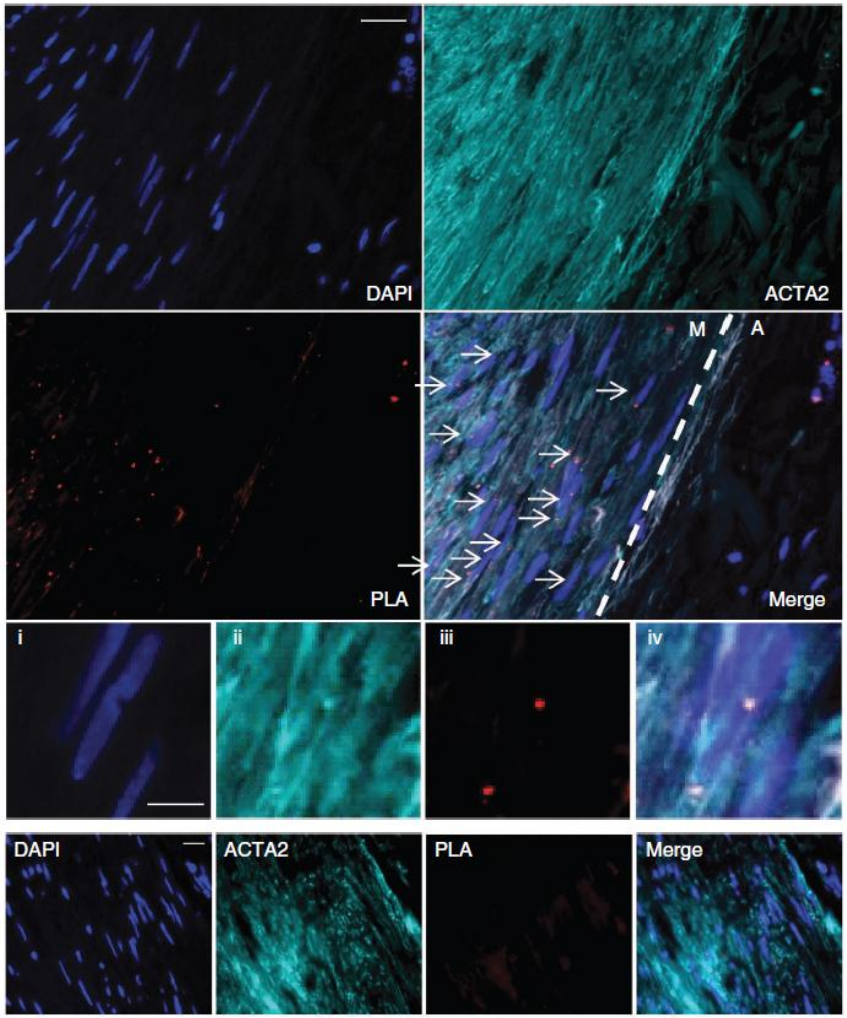
SMC-eYFP$^{+/+}$ mice



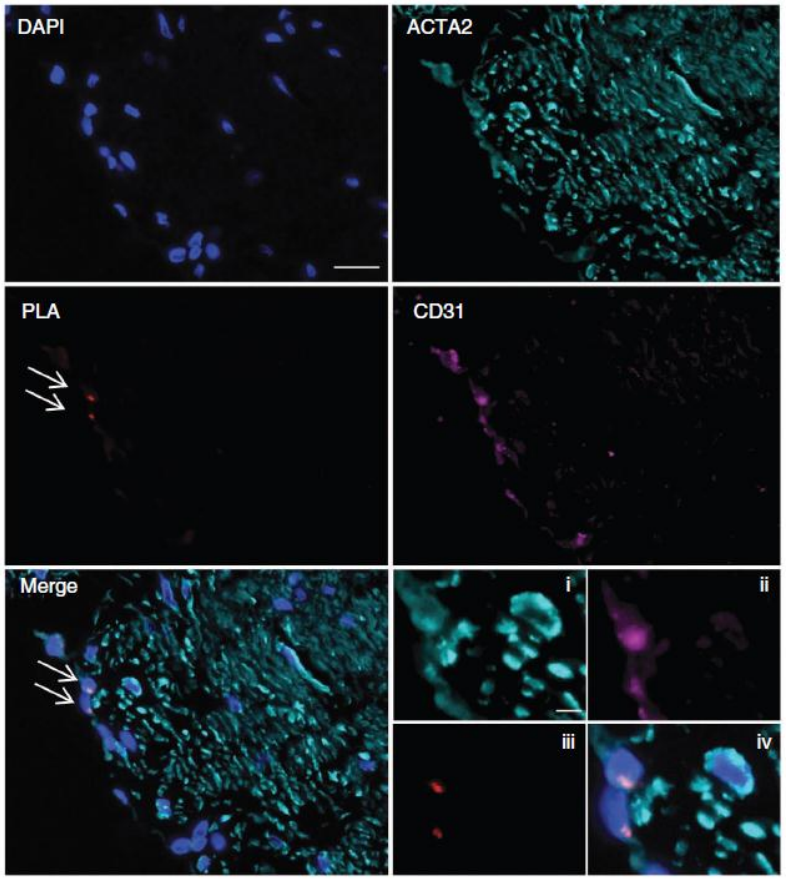
→ ISH-PLA could be adapted to additional gene loci

ISH-PLA analysis of human carotid artery sections

***MYH11* H3K4me2**



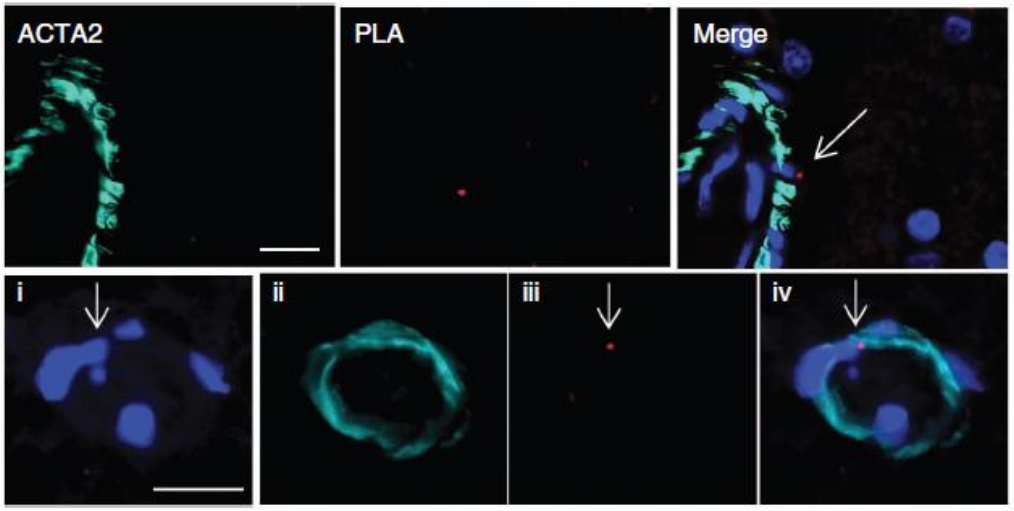
***MYH11* H3K27me3**



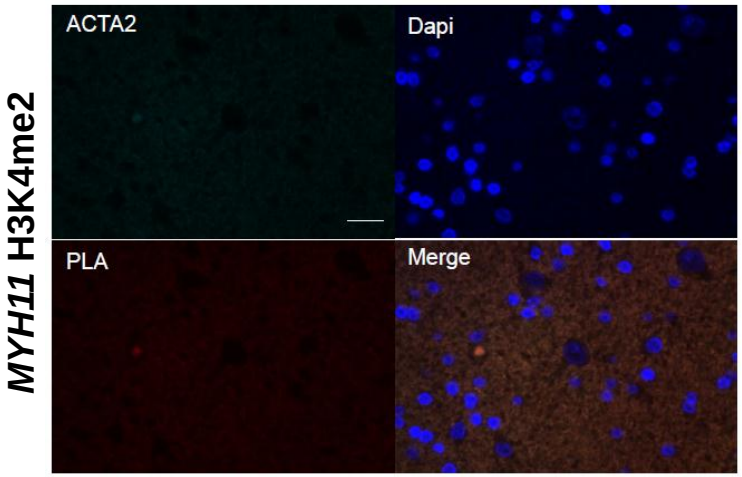
→ ISH-PLA could be adapted to additional histone modifications

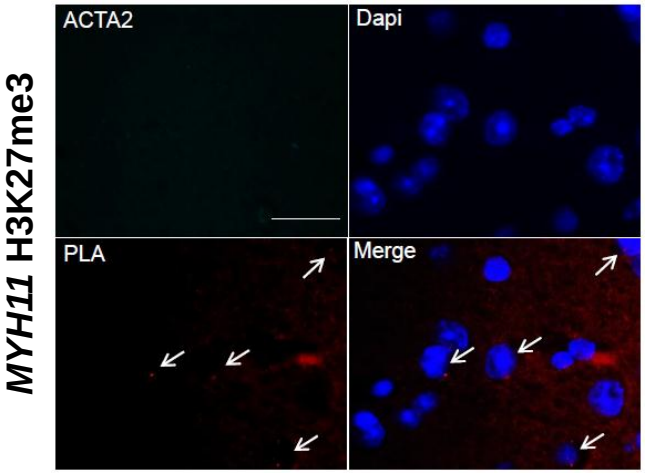
ISH-PLA analysis of human brain sections

ACTA2+ SMCs



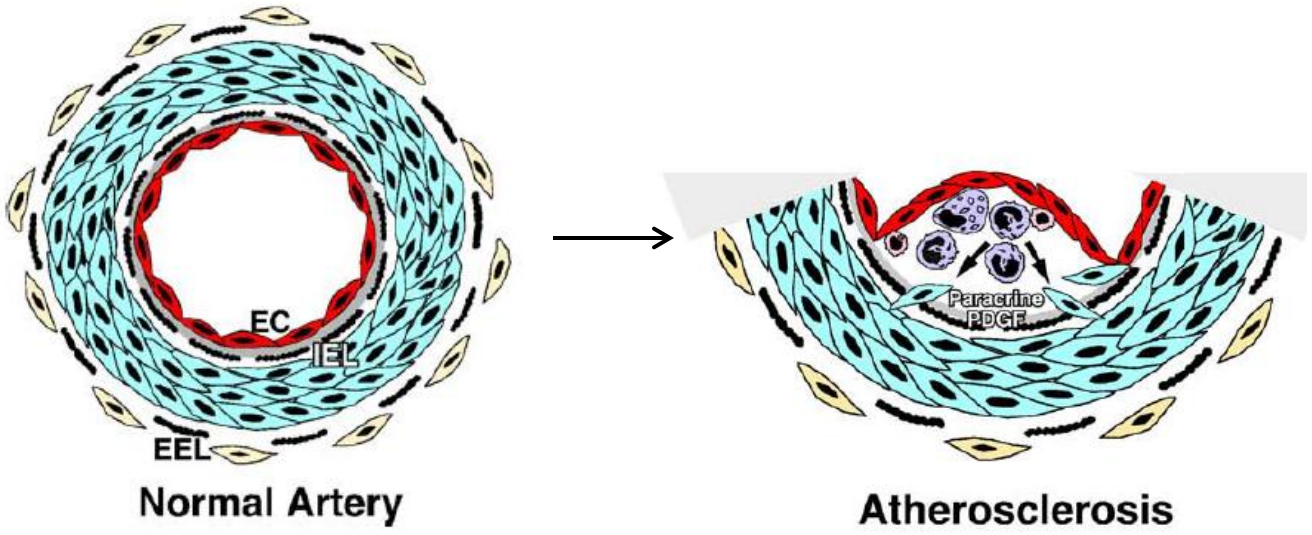
Non-SMCs





⟶ ISH-PLA could be adapted to additional tissues

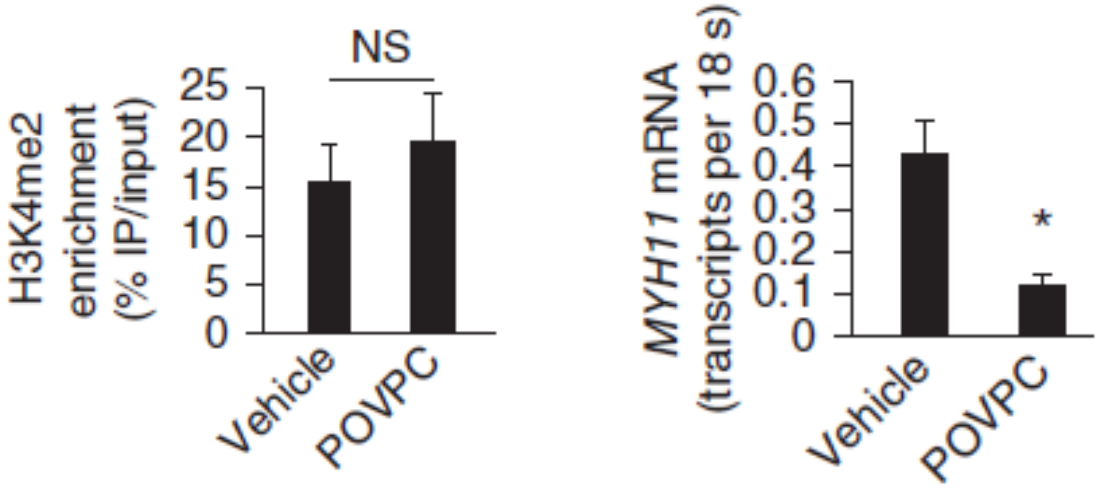
PDGF induces phenotypic switching of SMCs



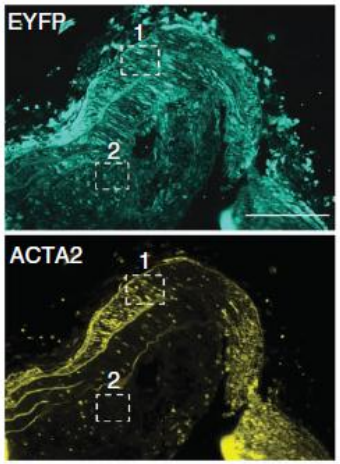
Elaine W Raines, *Cytokine & Growth Factor Reviews 2004*

- marked reductions in SMC marker expression
- reduced H4 acetylation

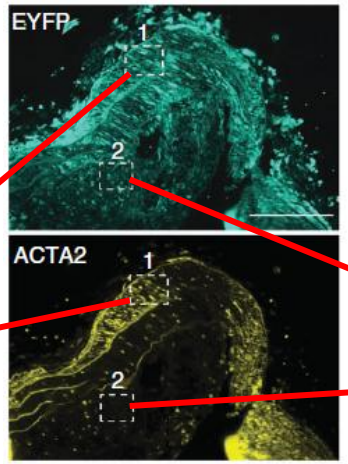
SMC phenotypic switching with the pro-atherogenic oxidized phospholipid POVPC



Phenotypically modulated SMCs (EYFP+ MYH11−) in lesions of SMC-*EYFP*+/+ *ApoE*−/− mice

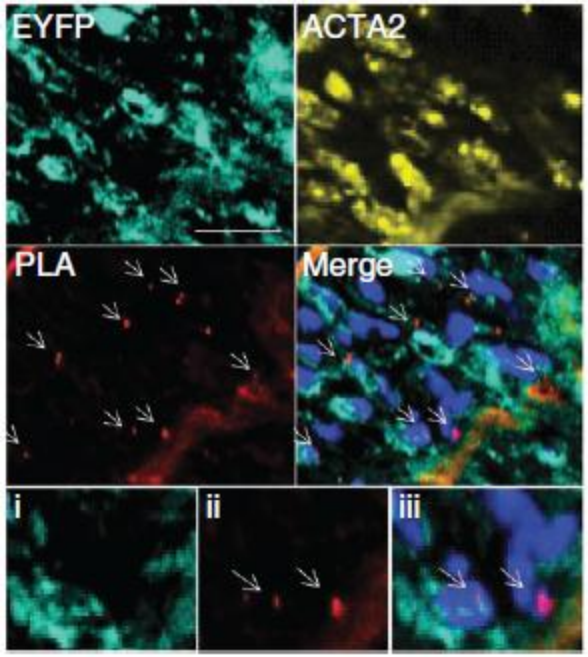


Phenotypically modulated SMCs (EYFP+ MYH11−) in lesions of SMC-*EYFP*+/+ *ApoE*−/− mice

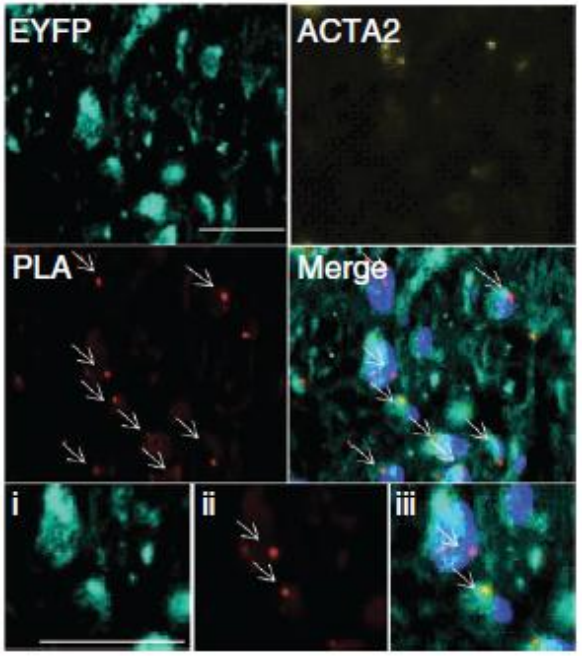


medial SMCs

SMC-derived cells

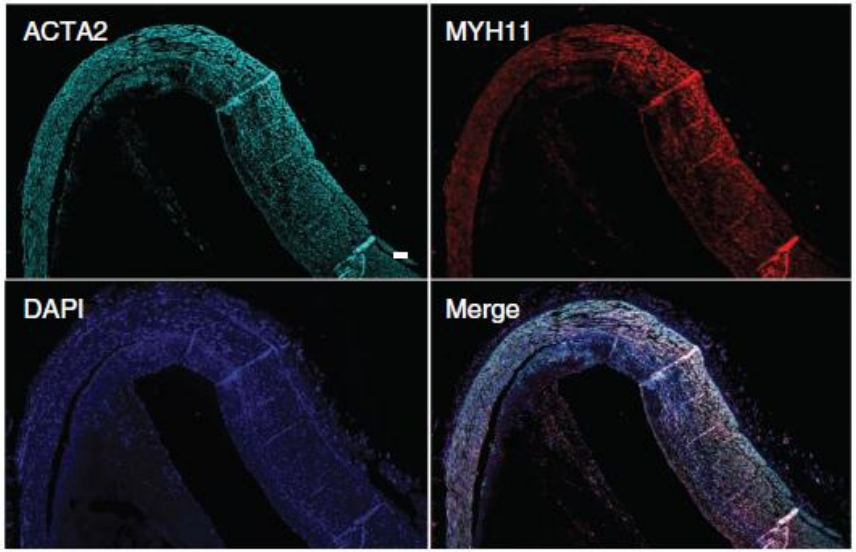


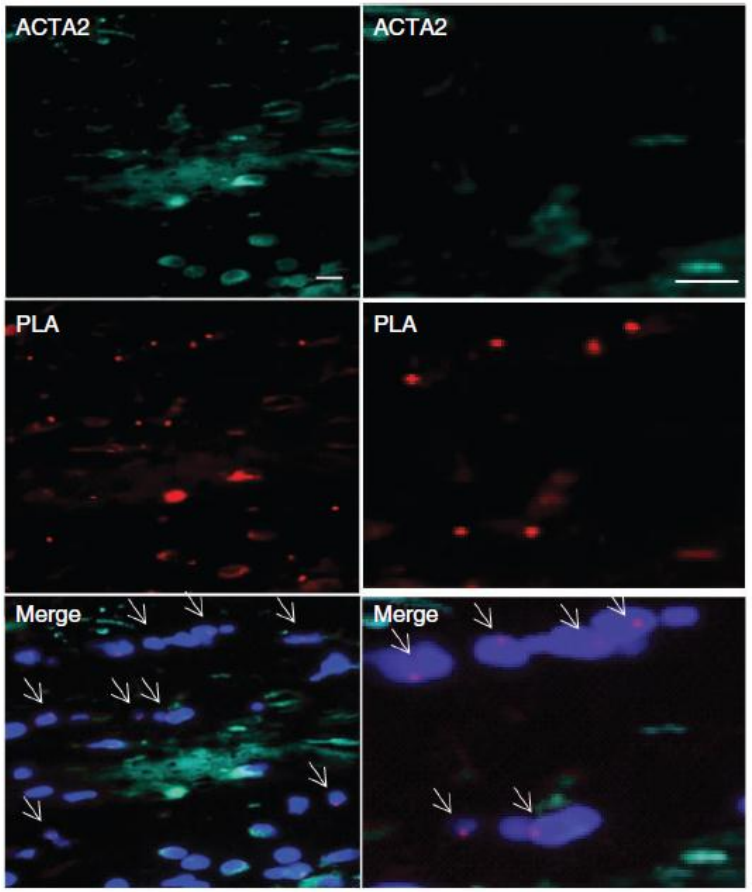
ACTA2+ EYFP+ and MYH11 H3K4me2 PLA+



EYFP+ and MYH11 H3K4me2 PLA+ but ACTA2−

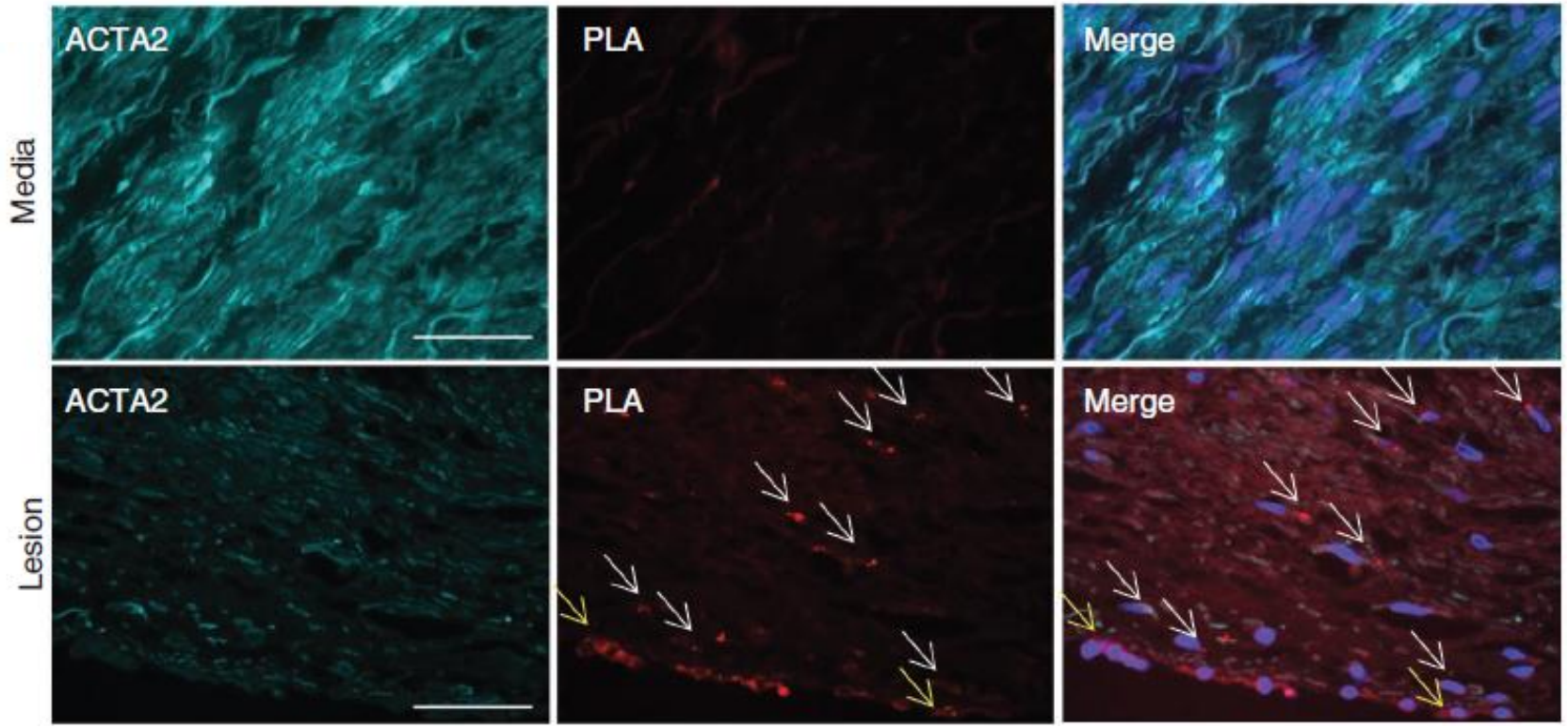
MYH11 H3K4me2 ISH-PLA of human coronary arteries





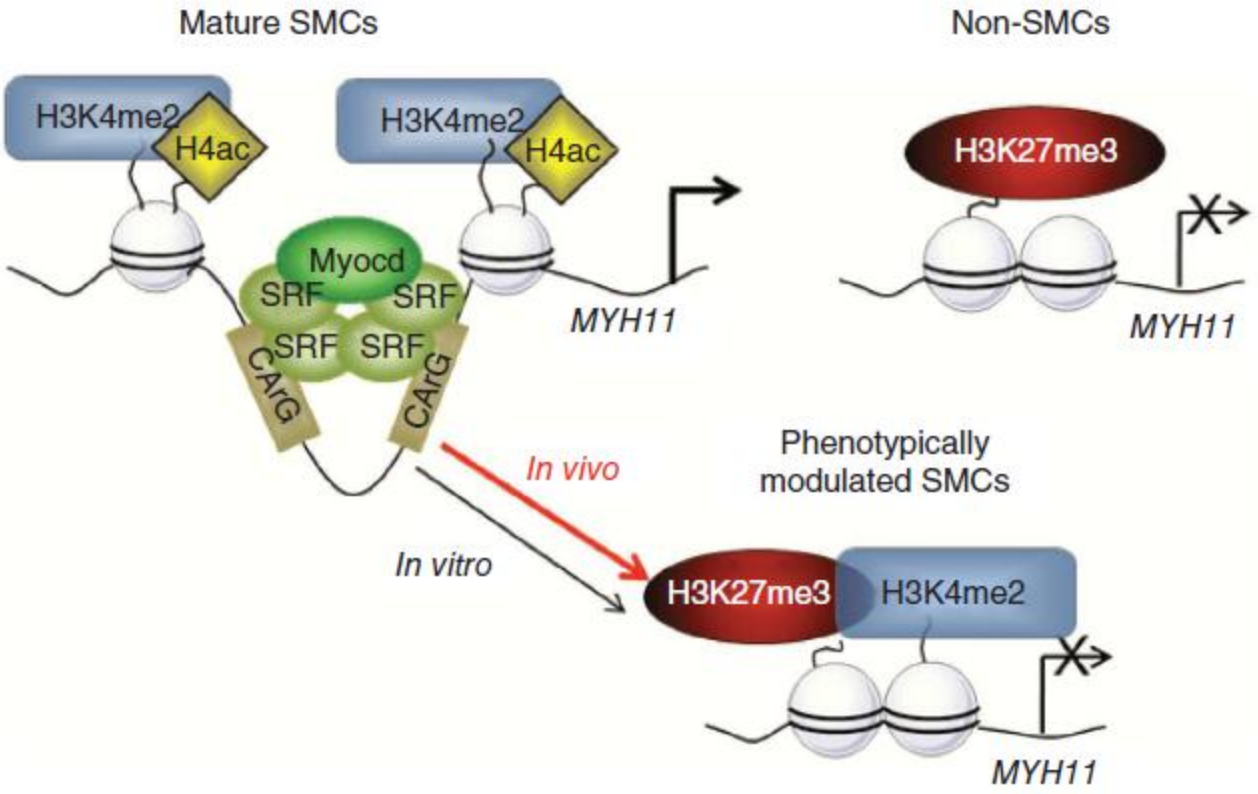
→ atherosclerotic lesions are ACTA2-, MYH11- and MYH11 H3K4me2+

MYH11 H3K27me3 ISH-PLA in human coronary atherosclerotic lesions



→ atherosclerotic lesions are ACTA2- and MYH11 H3K27me3+

epigenetic regulation on the *MYH11* promoter in mature SMCs, phenotypically modulated SMCs and non-SMCs *in vivo*



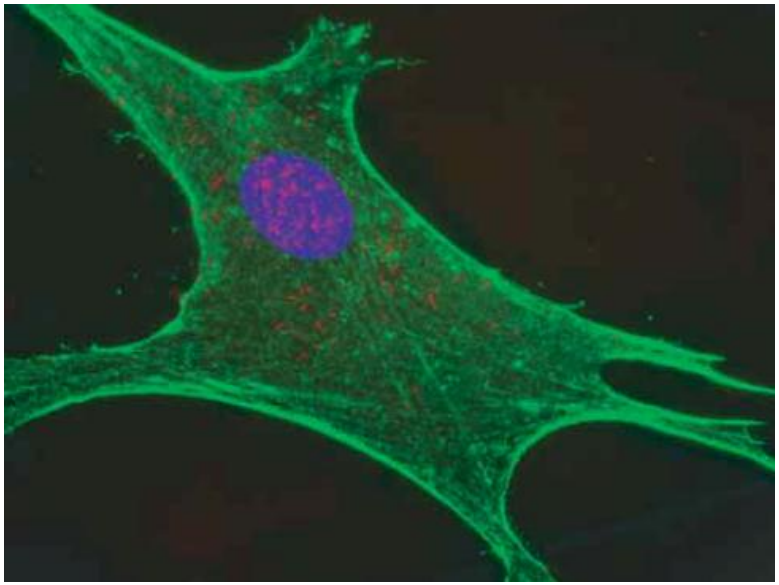
conclusion

- ISH-PLA method can reliably and specifically detect histone modifications at specific gene loci in single cells in human and mouse tissue sections
- identification of a cell type– and locus-specific histone modification in cells *in vivo* within intact tissue sections in a complex multicellular tissue specimen
- H3K4me2 of the MYH11 gene locus represents a unique and specific epigenetic signature of cells of the SMC lineage *in vivo*
- PLA methodology is easily adaptable to multiple gene loci and histone modifications

↓

methology has promise for broad applications in the study of epigenetic mechanisms in complex multicellular tissues in development and disease

Thanks for your attention



(O. Söderberg et al., Nature Methods 2006)