Quantitative proteomic analysis of integrin adhesion complex dynamics

aculty of __ife Sciences

Adam Byron

Wellcome Trust Centre for Cell-Matrix Research Faculty of Life Sciences University of Manchester

7th Joint BSPR/EBI Proteomics Meeting, Cambridge

Adam Byron



Introduction

- Integrins as adhesion receptors
- Challenges in the field

Isolation of adhesion complexes

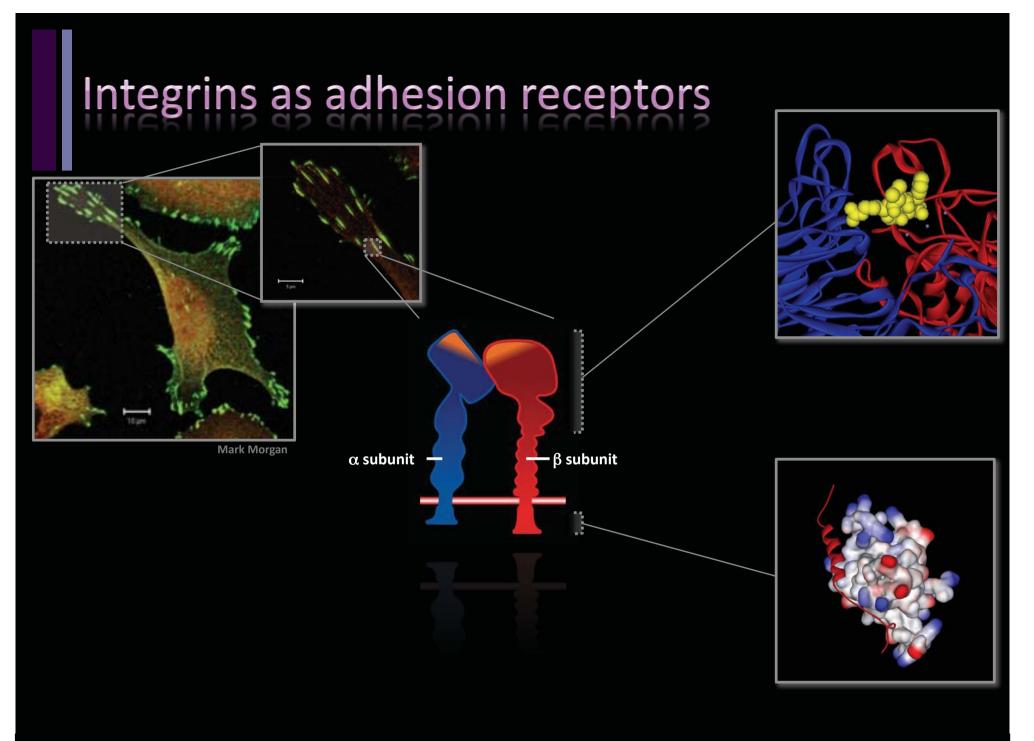
- Methodology
- Global analysis and candidate discovery

Current challenge

- Analysis of adhesion complex assembly

Ongoing work

- Activation state-dependent signalling



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Challenges in the field

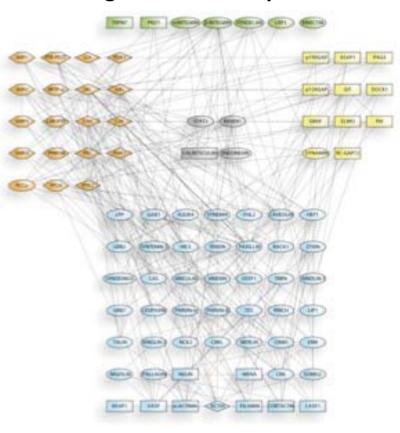
Integrin ligands

Humphries, Byron & Humphries (2006) J. Cell Sci. 119: 3901-3903

- Cell-type specificity
- Ligand specificity

- Stoichiometry
- Spatiotemporal dynamics

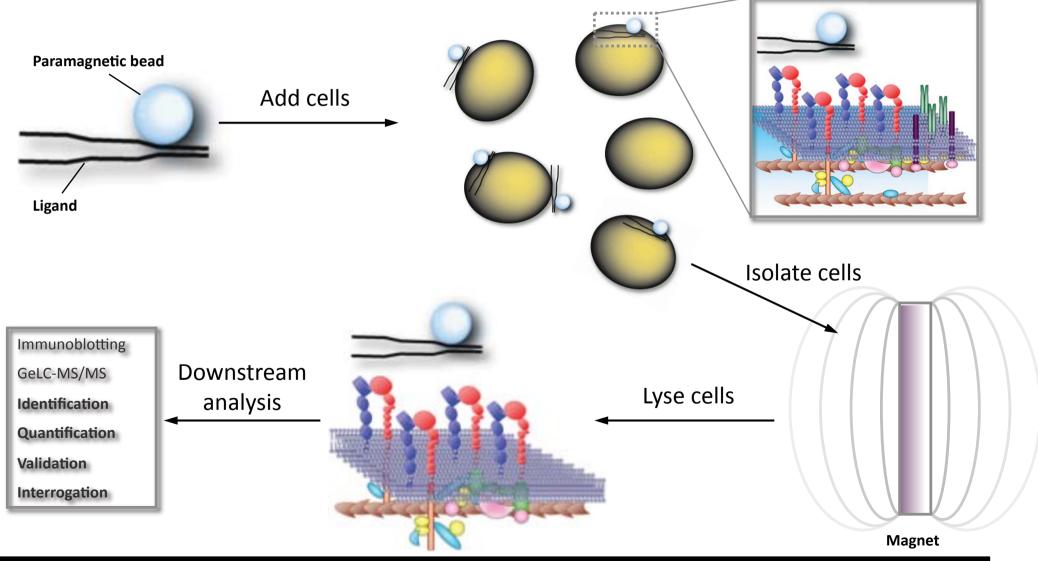
Integrin-associated proteins



Zaidel-Bar et al. (2007) Nat. Cell Biol. 9: 858-867

- Network modularity
- Network regulation

Isolation of integrin adhesion complexes

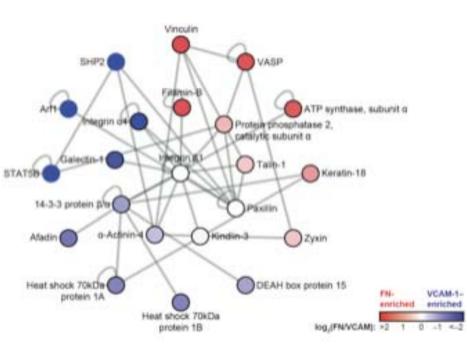


Ligand-induced interaction networks

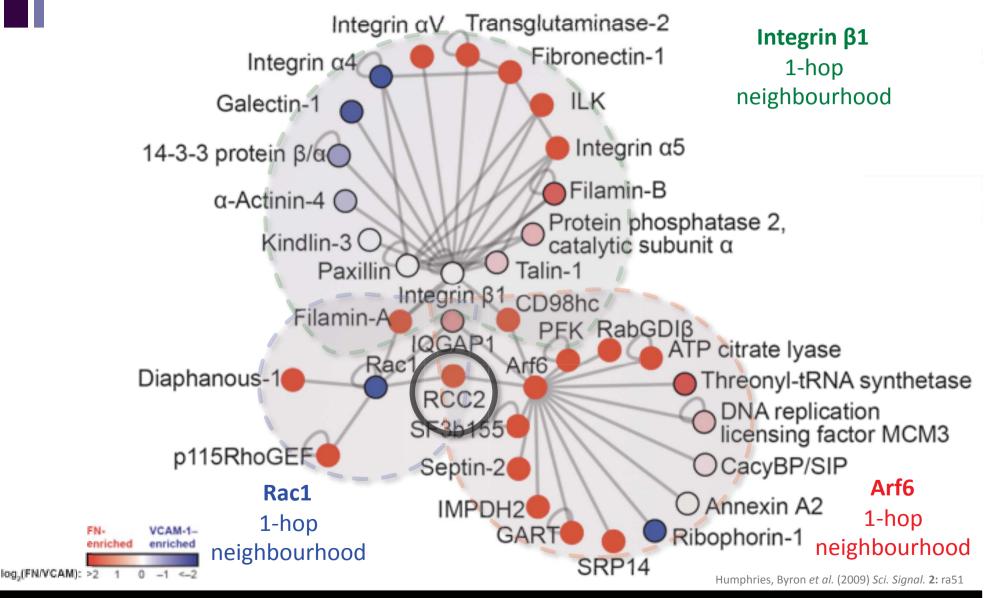
Fibronectin-induced network

(affyin be) Glutathione S-transferase x1 Captomer protein complex, subunit y Nucleoporin 205kDa Protessome, subunit 81 Protein phosphatase 1, Heat shock 70kDa satalytic subunit o protein 1A FUSE-binding protein-Heat shock 70kDa & riferacting repressor protein 18 Vacuolar protein DEAH box sorting 26 homolog A polypeptide 15 protein (\$/g 14-3-3 protein y Zyxin Eukaryotic translation elongation factor 2 Aldolase-A Glutathione S-transferase n Keratin-18 ATP synthase. Hyaluronan-binding protein 1 subunit a votein phosphillase 2. Annexin A1 catalytic aubunit of Casein kinase 2, subunit q1 Vinculin Coffin-1 Heat shock 70kDa protein 5 Acetyl-CoA carboxylase o 14-3-3 protein £ Splicing factor, arginine/serine-rich 3 FN-VCAM-1-Elongation factor Tu enriched enriched GTP binding domain log.(FN/VCAM): >2 1 0 -1 4-2 containing 2

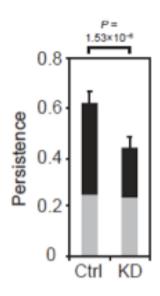
VCAM-1-induced network

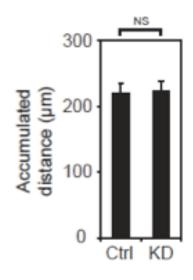


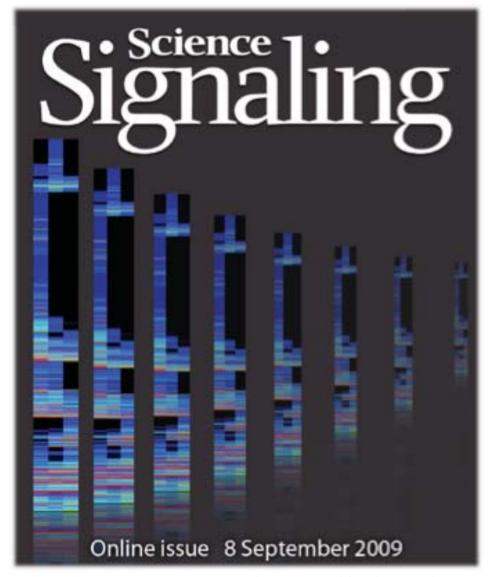
Links to GTPase signalling modules



RCC2 modulates directional cell migration





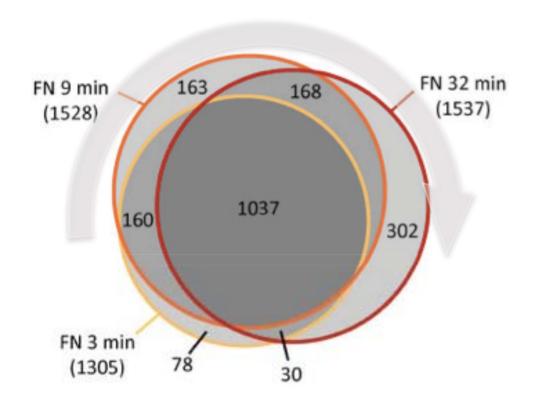


Humphries, Byron et al. (2009) Sci. Signal. 2: ra51

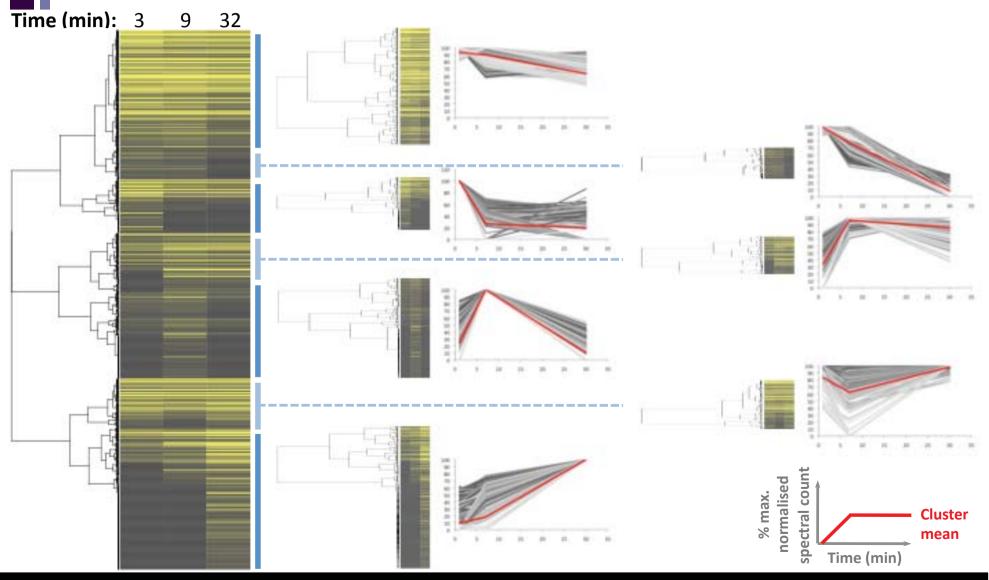
Current challenge

Dynamics of adhesion complex assembly

Adhesion complex assembly



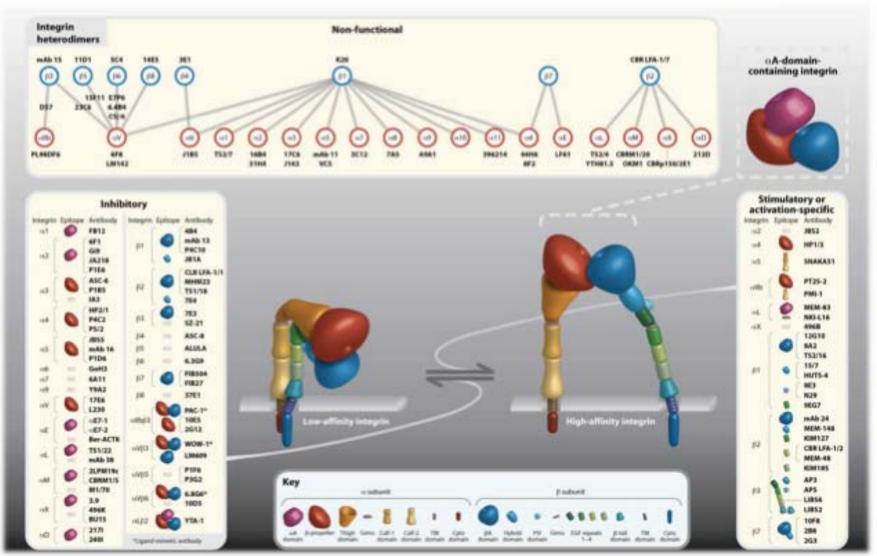
Adhesion complex assembly profiles





Activation state-dependent signalling

Activation state-dependent antibodies



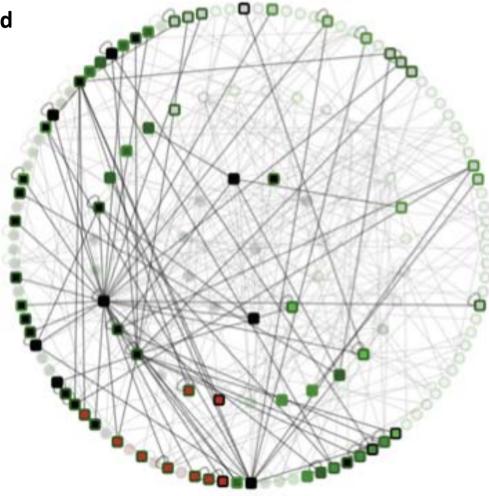
Byron et al. (2009) J. Cell Sci. 122: 4009-4011

Activation state-dependent networks

Integrin β1

3-hop neighbourhood

- active integrin



Summary

Methodology

- Isolation of specific integrin-associated protein complexes
- Order of magnitude more complex than previously thought

Ligand-specific adhesion complexes

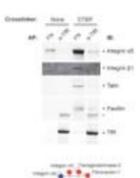
- Surprising variation in scale and composition
- Discovery of a novel regulator of integrin signalling

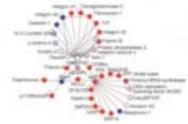
Adhesion complex assembly

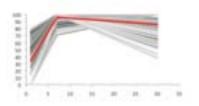
- Complexes form within 3 min of ligand induction
- Core complex matures with distinct temporal profiles

Activation state-dependent signalling

 Antibody-mediated activation appears to mimic early phase of ligand-induced activation









Acknowledgements

Cell biology/ biochemistry

Martin Humphries

Jon Humphries

Janet Askari

Mark Bass

Sue Craig

JIS

David Knight

Stacey Warwood

Emma Keevill

Informatics/ IT Julian Selley

Adam Huffman

John Pinney (Imperial College London)

Andrew Garrow (Unilever)

Ticroscopy

Jane Kott











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