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Alternative cellular roles for proteins identified using proteomics

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The recent report by Grigera et al. [1] provided interesting new data on the ability of the actin-binding protein cortactin to associate with regulator of chromosome condensation-2 (RCC2; also known as TD-60), a component of the mitotic machinery [2]. Owing to the localisation of cortactin to the equatorial plane of dividing cells, the authors inferred that its interaction with RCC2 was functionally important in mitosis. This may be the case, but the data also support an alternative interpretation that RCC2 may play a role in cortactin-mediated branched actin regulation during cell movement.

Cortactin promotes actin nucleation and stabilises F-actin, and thus it regulates many cell motility processes, such as focal adhesion assembly, lamellipodial protrusion, vesicle transport and endocytosis [3], although the full gamut of cortactin function is incompletely understood. Cortactin strongly localises to the leading edge of migrating cells, where it is phosphorylated, and signalling to small guanosine triphosphatases (GTPases), such as Rac1, plays an important part in its function [4,5]. Engagement of integrin adhesion receptors leads to activation of small GTPases, which play a central role in the modulation of cell adhesion complexes [6,7]. Using a proteomic approach, we showed that specific integrin adhesion complexes contain RCC2 (Fig. 1) [8–10]. Moreover, we demonstrated that, through precise restriction of Rac1 and Arf6 GTPase activity, RCC2 controls directional cell motility [8,9,11]. Indeed, our findings suggest that, rather than acting as a putative guanine nucleotide exchange factor and activating Rac1, RCC2 limits the activation of Rac1.

Together with the work of Grigera et al. [1], our data indicate an intriguing overlap between molecules implicated in cell adhesion processes and RCC2 function. Thus, the data reported by Grigera et al. [1] do not exclude the possibility that RCC2 interacts with cortactin as a component of the cell migration machinery [8,9] or indeed of integrin adhesion-regulated mitosis [12,13]. A current lack of data reporting the colocalisation of RCC2 with cortactin prevents a complete understanding of the interaction between these molecules, but further study of their contributions to cell behaviour is warranted.

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The works discussed above exemplify the discovery of unexpected cellular roles for proteins; such examples continue to increase as more data-driven proteomic studies appear in the literature. The consequence, in the postgenomic era, is that a single gene cannot be correlated with a single protein function. Proteins may possess multifunctionality within a single polypeptide chain, known as “moonlighting” [14], or as different splice variants. Therefore, understanding the context of protein interactions is critical to gaining mechanistic insight into protein function. Many MS-based proteomic studies rely on analysis of cell or tissue lysates, in which cellular context (e.g. subcellular localisation or cell type-specific expression) has been eliminated. Furthermore, many analyses of proteomic data use protein interaction networks as a tool for data interrogation. Such analyses lever the wealth of knowledge about all reported protein interactions and can be incredibly informative, including by suggesting alternative functions for proteins [15], but these datasets should be interpreted in the context of their derivation and the methodological limitations. Reported interactions in large-scale interaction networks have usually been amalgamated from data from multiple conditions, cell types and subcellular locations. With a lack of cellular context, proteins can appear to be promiscuous binders, whereas, in a cell, they may bind to distinct partners under restricted conditions. Accordingly, to achieve mechanistic insight into protein function, the analysis of protein interaction networks should rarely be the final interpretation of MS data, but instead be the springboard for further experimentation. Overall, data-driven MS-based proteomic and bioinformatic analyses, within the framework of hypothesis-driven research, provide a valuable opportunity for the discovery of new cellular roles for proteins, and will continue to provide surprising results about biological systems.

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Abbreviations

GTPase	guanosine triphosphatase
RCC2	regulator of chromosome condensation-2.

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Fig. 1.

Proteomic analysis of molecular interactions can reveal alternative cellular roles for proteins. RCC2 (red circle, or node, in interaction network) is a passenger protein with a role in cell division [2]. Proteomic and bioinformatic analyses of integrin adhesion complexes identified RCC2 at the intersection between $\beta 1$ integrin, Rac1 and Arf6 subnetworks (molecular interactions shown as light grey lines, or edges, in interaction network) [8]. Moreover, cell biological analysis revealed an unexpected role for RCC2 in directional cell migration [8]. Proteomic analysis of proteins associated with the actin-binding protein cortactin identified RCC2 (light blue line in interaction network) [1]. The contribution of the association between cortactin and RCC2 to cell division or cell migration remains to be investigated.