Primer

Adhesion signalling complexes

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Intercellular communication in metazoa not only requires autocrine, paracrine and exocrine signalling systems, but it also relies on the structural and positional information encoded in extracellular matrices (ECMs). Most cells in tissues are structurally and functionally integrated with their surrounding ECM in a highly organised manner involving thousands of dynamic connections. On the intracellular face of these linkages, adhesion receptors — principally integrins and syndecans — link the cytoskeleton to the plasma membrane and compartmentalise cytoplasmic signalling events, whereas at the extracellular face the same receptors direct and organise the deposition of the ECM itself. Adhesion receptors transduce mechanical force bidirectionally across the plasma membrane by tethering variably deformable ECMs to the contractile cytoskeleton (Figure 1), and they translate the topography and composition of the ECM into chemical signals that determine behaviour. The membrane-proximal functions of adhesion receptors in turn trigger distal processes within cells, such as alterations in the direction of cell movement and the regulation of gene transcription, and long-range effects outside cells, such as the construction of ECM networks and consequent shaping of higher-order tissue structure. Given the diverse and fundamental roles attributed to adhesion, it is understandable that adhesion receptor engagement has been reported to alter the flux through virtually all major signalling pathways.

Cell adhesion receptors

It has been estimated that ~500 genes encode the glycoproteins and proteoglycans that combine to form ECM fibres and networks. Despite the diverse range of ECM components, cellular recognition is mediated by a small number of receptor families, amongst which integrins and syndecans are dominant. In mammals, the integrin family comprises 24 $\alpha\beta$ heterodimers that result from selective non-covalent associations between 18 α and 8 β subunits. The syndecan family is much simpler, with four members in mammals. All integrin and syndecan subunits are composed of large extracellular and typically small cytoplasmic domains. The specificity of syndecan–ligand binding may be subtly modulated by glycosaminoglycan post-translational modification, but this is not well understood. By contrast, the particular $\alpha\beta$ combination of integrin extracellular domains clearly determines ligand-binding specificity.

Adhesion is required not only for anchorage, but also for mediating a diverse range of phenotypic responses to the cellular microenvironment. Most attention has been focused on determining how adhesion receptors regulate chemical signalling by controlling the spatiotemporal assembly of enzymes and adaptors. However, there is emerging evidence that cell–ECM interactions also act as sites of mechanotransduction, transmitting short-range tensile and elastic force across the plasma membrane and interpreting long-range alterations in tissue flow. Historically, there has been substantial interest in understanding the effects of physical force on cell behaviour, but progress has been limited. Recently, however, important new insights have been obtained into the role of integrins as mechanosensitive receptors that display catch-bond behaviour. In addition, cytoskeletal adaptors that undergo force-dependent activation or provide mechanosensitive bridges within the cytoskeleton have been identified.

Adhesion complexes

In cells adherent to ECM proteins *in vitro* or *in vivo,* adhesion signalling

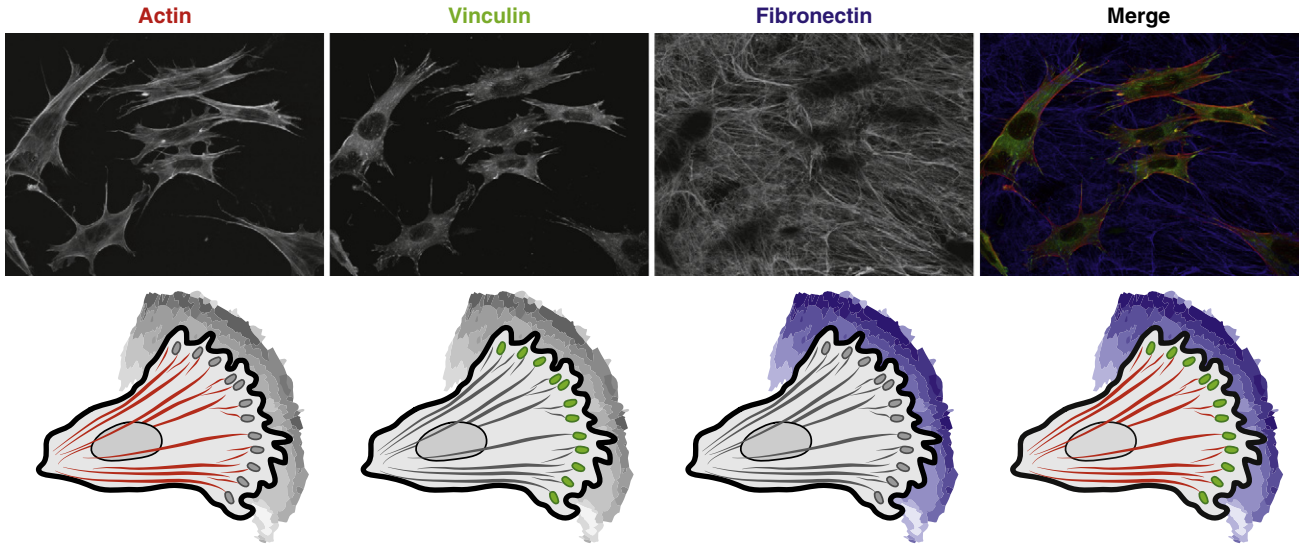

Figure 1. Adhesion complexes integrate the ECM with the actin cytoskeleton.
Integrin-mediated adhesion complexes regulate dynamic integration of the fibrillar extracellular environment with the contractile cytoskeletal machinery, promoting bidirectional application of force, cell migration and ECM remodelling. Fibroblasts plated on cell-derived matrices (blue, fibronectin) exhibit bundled actin fibres (red) that terminate at multimolecular adhesion complexes (green, vinculin) at sites of cell–ECM interaction.

complexes are distributed focally rather than diffusely and are manifested as asymmetric patches and stripes. Detailed morphological and functional analyses have defined three major forms of adhesion contact: focal complexes, focal adhesions and fibrillar adhesions, all of which are associated with the contractile polymers of the cytoskeleton. Each class of contact is formed sequentially and disrupted as cells translocate in a process that appears to be highly conserved between different cell types. Initially, focal complexes form at the posterior edge of ruffling membrane, where they anchor the short filopodial struts and lamellipodial meshes of actomyosin that mediate membrane protrusion. When protrusion ceases, or the lamellipodium retracts, focal complexes transform into larger focal adhesions, which provide a more robust anchorage via transcellular actomyosin-containing stress fibres. In turn, focal adhesions evolve into fibrillar adhesions, which are the major sites of fibronectin matrix deposition and remodelling.

The physiological role of adhesion complexes has been the subject of much debate, primarily because our understanding of the maturation, dynamics and function of adhesion complexes has relied on analyses *in vitro*. Since cells plated onto tissue-derived 3D fibrillar matrices form adhesion plaques that differ in size and composition from those observed on 2D substrates, researchers have questioned whether focal adhesions actually exist in metazoa. Nevertheless, electron microscopy and immunofluorescence imaging have identified adhesion-like structures *in vivo* at sites of cell–ECM interaction, such as at the end of actin filaments at the interface between endothelial cells and the basement membrane, in myotendinous junctions formed by skeletal muscle cells and in embryonic mesenchymal matrices. Moreover, processes known to modulate focal adhesion dynamics, including GTPase regulation and adhesion receptor crosstalk, are essential for directing cell migration *in vivo,* and physical interactions with ECM *in vivo* are essential for controlling cell survival. Consequently, genetic ablation of many adhesion receptors results in embryonic lethality or severe perturbation of processes associated with cell migration and ECM remodelling (e.g. wound healing, immune surveillance and angiogenesis). While it is clear that regulation of cell–ECM adhesion is essential for multicellular life, it is likely that adhesion complexes *in vivo* are subject to exquisite spatiotemporal regulation. It has been suggested that adhesion complexes defined *in vitro* may be enhanced versions of adhesions *in vivo*. Currently, technological limitations prevent detailed analysis of adhesion complex dynamics *in vivo*; however, by studying the processes that regulate focal adhesion formation and turnover *in vitro,* it is possible to dissect the molecular events that regulate cell–ECM engagement *in vivo* where they are probably subject to stringent spatial and temporal constraints.

Regulation of adhesion complex dynamics (i.e. formation, stabilisation, maturation and disassembly) is required to orchestrate locally the bidirectional application of force between the actin cytoskeleton and the ECM. Thus, precise coordination of adhesion turnover is critical for efficient cell migration and cell-mediated ECM remodelling. Adhesion contact dynamics can be controlled by GTPase activity, integrin activation, receptor trafficking, heterodimer-specific engagement of integrins and microtubule targeting. Moreover, many of these molecular or cellular events can influence each other directly (Figure 2). The Rho GTPases are a family of monomeric guanine-nucleotide-binding proteins that function as molecular switches and play a central role in the regulation of actin polymerisation and the generation of intracellular tension. Rac1 activity promotes formation of nascent focal complexes, whereas RhoA promotes maturation of focal complexes to focal adhesions, formation of bundled actin stress fibres and, by promoting translocation of α5β1 integrin, generation of fibrillar adhesions. Importantly, the localised activation of Rho GTPases is regulated by engagement of both integrins and syndecans. Syndecan-4 ligation is absolutely required for the transient activation of Rac1 during spreading on fibronectin, whereas the transient p190RhoGAP-dependent suppression of RhoA activity is mediated by convergent signals from α5β1 and syndecan-4.

Interestingly, differential engagement of the fibronectin-binding integrins α5β1 and αvβ3 can coordinate adhesion complex dynamics and biomechanical properties: α5β1 promotes adhesion complex turnover but can support ECM tension, whereas αvβ3 expression stabilises focal adhesions and, upon cytoskeletal association, promotes mechanotransduction. Thus, mechanisms that coordinate the spatial compartmentalisation of integrin and syndecan engagement have the capacity to fine-tune adhesive complex dynamics in response to the extracellular environment. Adhesion receptor trafficking, endocytosis and recycling back to the membrane could serve such a purpose. Under basal conditions, α5β1 and αvβ3 have the capacity to be recycled through the same pathway, whereas in response to certain growth factor or ECM stimuli, α5β1 and αvβ3 are driven along reciprocal and antagonistic recycling pathways that influence heterodimer-specific integrin engagement, GTPase activity and cell migration. Thus, within a migrating cell, trafficking of adhesion receptors to discrete regions of membrane spatially restricts the initiation of signalling events and can potentially dictate adhesion complex dynamics.

Microtubules are dynamic cytoskeletal polymers that target sites of ECM engagement and promote focal adhesion disassembly during cell migration. It is now clear that microtubules regulate adhesion complex disassembly, at least in part, by promoting integrin endocytosis. However, microtubules also have the capacity to promote Arf6-dependent recycling of internalised membrane microdomains to the membrane in order to promote and compartmentalise adhesion-dependent Rac1 activity.

The apparently high level of crosstalk between the mechanisms that regulate adhesion contact dynamics (Figure 2) highlights the fact that these systems need to be tightly and precisely regulated. The next big challenge will be to determine how these processes and signalling networks are integrated both spatially and temporally to coordinate mechanotransduction and mechanosensation during cell migration and ECM remodelling.

Molecular complexity of adhesion sites

Over 150 components of adhesion signalling complexes have been described, and based on their reported pairwise interactions, a hypothetical integrin ‘adhesome’ has been created.

Despite the enormous value of this literature-curated view of adhesion complexes, insights into the molecular composition of focal complexes, focal adhesions and fibrillar adhesions are currently limited, and the stoichiometry, modular sub-structure, turnover and dynamic relationships of molecules in natural adhesion complexes are not understood. A hierarchy of recruitment of signalling and cytoskeletal molecules to integrins has been demonstrated using immunocytochemical approaches, but as yet there is little evidence for ligand- or agonist-specific differences in the composition and/or organisation of this hierarchy.

A characteristic feature of most integrin receptors is their ability to recognise a wide variety of extracellular ligands. Furthermore, many ECM and cell-surface adhesion molecules bind to multiple integrins. Integrin engagement elicits signalling responses that are specific to the integrin–ligand combination, although a comprehensive understanding of this specificity is currently lacking. Nonetheless, ligand-specific signals are transduced intracellularly by the recruitment of large, multiprotein adhesion complexes, the composition of which varies depending on the signal. There exists, therefore, a combinatorially complex set of possible molecular outcomes at both the extracellular, ligand-binding domain and the cytoplasmic, adhesion-complex-binding domain of integrin receptors.

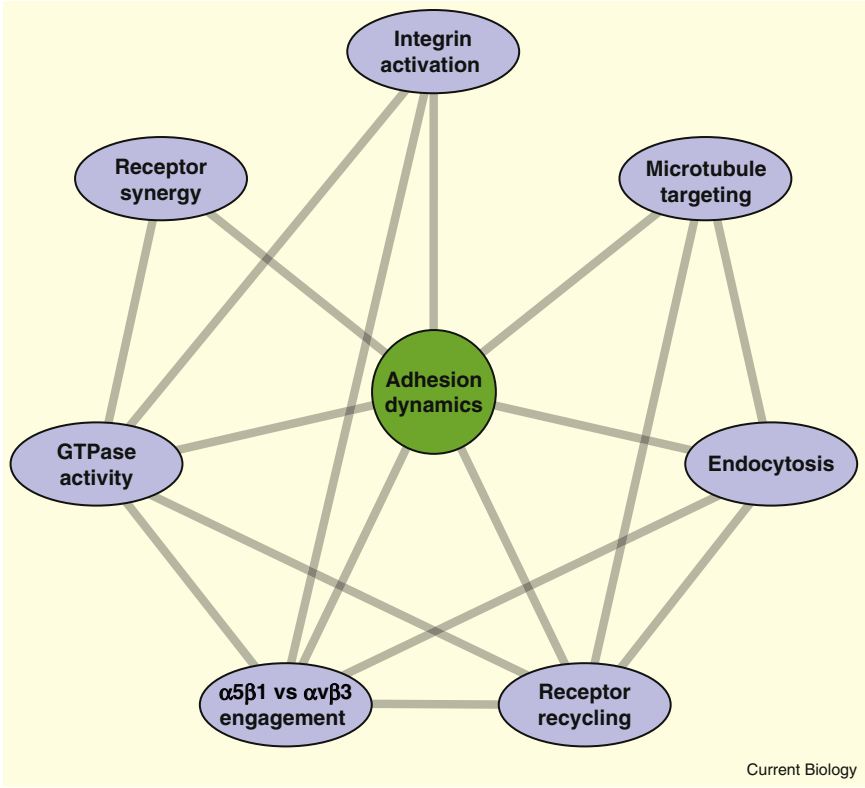

Figure 2. Regulation of adhesion complex dynamics.
Schematic representation of the different factors that can regulate focal adhesion dynamics (including GTPase activity, integrin activation, integrin–syndecan synergy, receptor trafficking, heterodimer-specific integrin engagement and microtubule targeting) and the potential for crosstalk between these different processes.

Spatiotemporal control of adhesion complexes

How is this complexity overcome *in vivo* to result in regulated cell behaviour? The answer largely lies in the spatial and temporal specification of adhesion complexes, the control of which can be considered at several scales, including the tissue, the cell and the adhesion complex itself.

For multicellular organisms, the combination of integrin expression pattern, integrin activation state and availability of ligand determines the cell–ECM interactions that occur in the context of the tissue. Integrins expressed by hematopoietic cells, for example, can bind to counter-receptors, such as intercellular adhesion molecule-1 (ICAM-1), presented by other cells in the vasculature. Moreover, during developmental cell movements, tissue renewal and wound repair, large-scale migration of cells over the course of hours or days results in the exposure of some cells to different ECM environments and hence to distinct integrin ligands. These spatial and temporal changes in integrin–ligand combinations provide context-specific cues to which cells respond by altering their shape, migration, proliferation and differentiation. The mechanisms of cell adhesion that precisely control the specification of niches or cell migration are not completely understood, however.

At the level of the adhesion complex, the network of proteins that define the multimolecular complex is probably considerably more expansive than previously thought. The list of reported components of adhesion complexes continues to grow, but these components are not all present in a complex at the same time. The specific combination of component parts is critical to determine the function of the complex, as indicated by the 'flavours' of adhesion complexes that are recruited to different integrin–ligand pairs (Figure 3) or the change in complex composition during adhesion site maturation. Another level of spatial restriction of molecules within adhesion complexes includes the modulation of protein–protein interactions by actomyosin contraction or enzymes, such as Src, which alters the diffusibility of components of adhesion complexes and may reveal new protein-binding sites. In addition, the bringing together of enzymes and substrates by scaffold proteins, such as paxillin, localises signalling events to adhesion complexes. The organisation of adhesion complexes at the plasma membrane has yet to be fully elucidated, although elements of the ultrastructural architecture of an adhesion site have recently been determined by cryo-electron tomography. On the basis of reported protein–protein interactions, the networks of components of adhesion complexes appear to be highly interconnected (Figure 3). The networks exhibit multiple alternative connections between components, which may provide structural or functional robustness to the specific complexes, emphasising their fundamental importance *in vivo*.

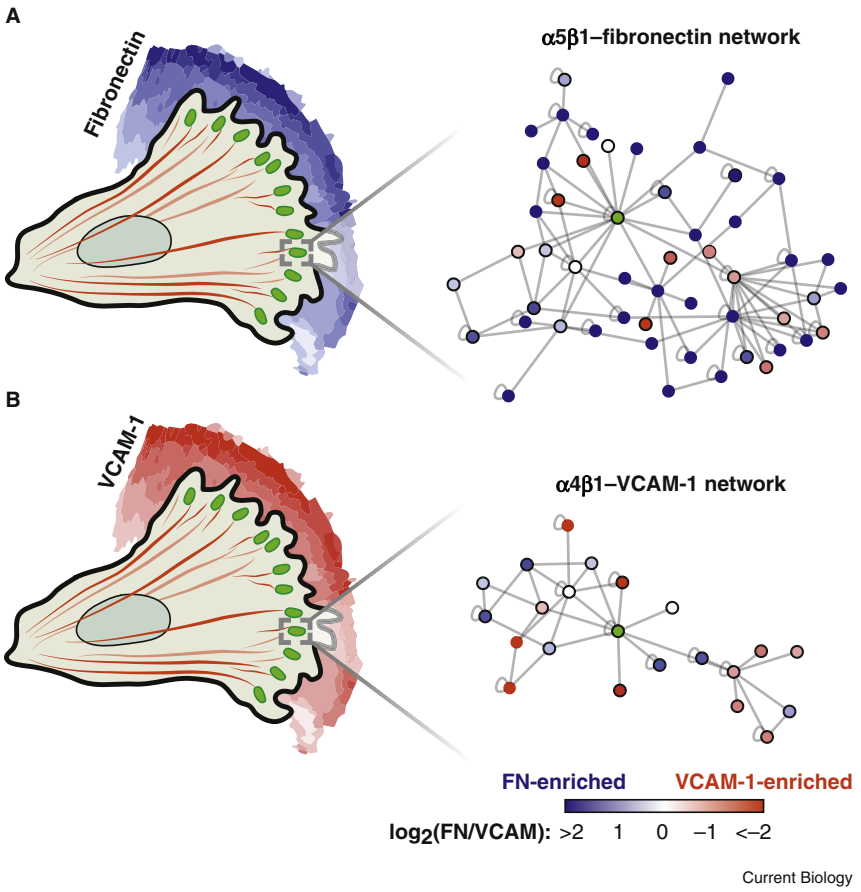

Figure 3. Molecular complexity of integrin adhesion complexes.
Engagement of different ECM ligands by a cell (left) results in the recruitment of specific adhesion complexes that vary dramatically in composition and scale (right). Proteins recruited to an α5β1–fibronectin complex (A) and an α4β1–vascular cell adhesion molecule-1 (VCAM-1) complex (B) as determined by mass spectrometry are displayed as interaction network models. Proteins (circles) are connected by potential protein–protein interactions (lines). Proteins are coloured according to their relative enrichment to fibronectin (blue) or VCAM-1 (red); β1 integrin is shown in green. Proteins identified in both integrin–ligand complexes have a black border. Only proteins within two path lengths of β1 integrin are displayed.

Consideration of all of these scales of complexity reveals that spatiotemporal context is central to the control of adhesion signalling. The specificity of the signal is encoded by the components of the adhesion complex, the availability of these components and their dynamics, while the coordination of the combined signalling of a heterogeneous population of receptor complexes may fine-tune the cellular phenotype. However, the resolution at which the control of adhesion signalling occurs is poorly understood, which makes the systems-level understanding of adhesion signalling a daunting but important goal for future research.

Mechanisms of regulation of adhesion complexes

How are the signalling control mechanisms regulated at sites of adhesion? Unlike growth factors, integrins lack intrinsic catalytic activity. Thus, enzymatic proteins form a significant proportion of the components of integrin-based adhesion complexes. Indeed, Src family kinases are essential to mediate integrin signal transduction but are largely dispensable for signalling from the platelet-derived growth factor receptor. An early event during integrin signalling is the tyrosine phosphorylation of the non-receptor tyrosine kinase focal adhesion kinase (FAK) in response to cell adhesion. Src, in coordination with FAK, promotes tyrosine phosphorylation of adaptor proteins, such as Shc, p130Cas and paxillin, and cytoskeletal proteins, such as α-actinin. These phosphorylation events can lead to the activation of multiple downstream signalling cascades, including phosphatidylinositol 3-kinase, phospholipase C and mitogen-activated protein kinase pathways. A negative feedback loop between FAK and phosphorylated paxillin has been suggested to regulate adhesion assembly, highlighting a potential mechanism for the regulation of adhesion turnover. Furthermore, α4β1 integrin can activate Src independently of FAK, resulting in phosphorylation of p130Cas, suggesting that different network connections in adhesion complexes can lead to the activation of common signalling pathways. The control of phosphorylation of scaffold proteins by the multiple tyrosine and serine/threonine kinases and phosphatases in adhesion sites also plays a role in the recruitment of GTPase-activating proteins and guanine nucleotide exchange factors for Rho GTPases.

Adhesion signalling crosstalk

It is becoming increasingly apparent that adhesion sites are regulated by crosstalk between integrin-associated complexes and several other receptor systems. Cooperation between integrins and syndecans is fundamental for precisely controlled cell migration. Syndecans act as adhesion receptors by engaging ECM molecules but also have the capacity to bind a large variety of growth factors and their receptors via covalently attached glycosaminoglycan chains to localise growth factor receptor signalling. Growth factors, such as fibroblast growth factor, can alter the expression of components of adhesion sites, and synergy between growth factor receptor and adhesion signals can occur both at the level of the receptors or the associated protein complexes. There is also interplay between integrins and receptors for cytokines, such as interleukin and prolactin, which activate Janus family tyrosine kinases that regulate downstream signalling events including gene expression. The cooperative, multireceptor organisation of adhesion signalling adds additional complexity to the regulation of cell adhesion, but the integrative dynamics of these synergies provides responsiveness and sensitivity to a system that must be exquisitely controlled. Thus, adhesion signalling does not occur as an isolated, linear cascade but instead as an interconnected network of control points. The precise nature of the modularity or hierarchy of the network control points remains to be determined.

Future perspectives

A large number of key players that associate with and regulate integrin-based adhesion complexes have been identified. Due to the molecular complexity and context dependence of cell adhesion, however, a comprehensive understanding of the protein networks involved has yet to be achieved. Recent advances in the isolation and proteomic analysis of adhesion complexes have demonstrated the potential of mass spectrometry-based proteomic approaches to enable global analyses of adhesion receptor-mediated processes. Such approaches, in combination with advanced microscopy, genomic sequencing and computational modelling, will ultimately pave the way to a quantitative, systems-level understanding of adhesion signalling.

Further reading

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Sex differences in chimpanzees' use of sticks as play objects resemble those of children

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Sex differences in children's toy play are robust and similar across cultures [1,2]. They include girls tending to play more with dolls and boys more with wheeled toys and pretend weaponry. This pattern is explained by socialization by elders and peers, male rejection of opposite-sex behavior and innate sex differences in activity preferences that are facilitated by specific toys [1]. Evidence for biological factors is controversial but mounting. For instance, girls who have been exposed to high fetal androgen levels are known to make relatively masculine toy choices [3]. Also, when presented with sex-stereotyped human toys, captive female monkeys play more with typically feminine toys, whereas male monkeys play more with masculine toys [1]. In human and nonhuman primates, juvenile females demonstrate a greater interest in infants, and males in rough-and-tumble play. This sex difference in activity preferences parallels adult behavior and may contribute to differences in toy play [1]. Here, we present the first evidence of sex differences in use of play objects in a wild primate, in chimpanzees (*Pan troglodytes*). We find that juveniles tend to carry sticks in a manner suggestive of rudimentary doll play and, as in children and captive monkeys, this behavior is more common in females than in males.

During 14 years of observation of the Kanyawara chimpanzee community in Kibale National Park, Uganda, we found that chimpanzees used sticks in four main ways: as probes to investigate holes potentially containing water or honey; during aggression, either as props in displays or as weapons (throwing or hitting) in aggression

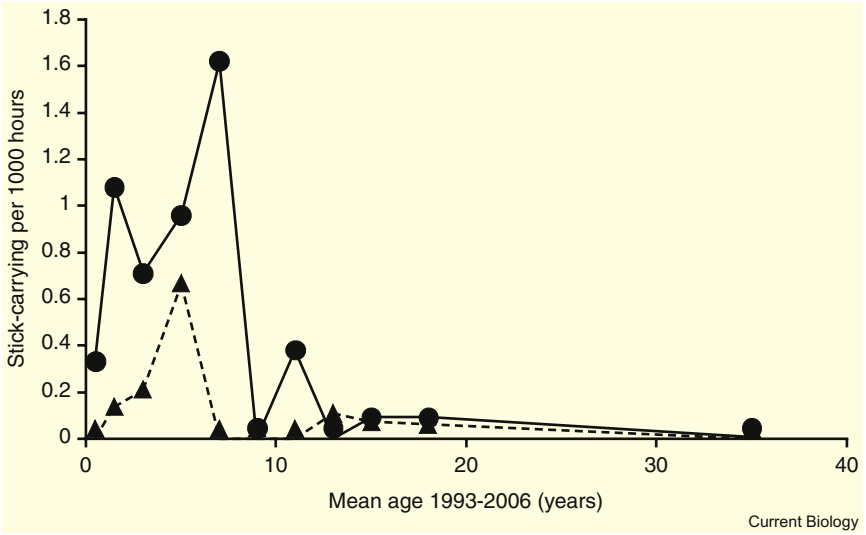

Figure 1. Age and sex differences in the rate of stick-carrying in chimpanzees.
Females: circles, solid line. Males: triangles, dashed line. Age categories referred to in the main text are infants (0–4.9 years (yrs)), juveniles (5–7.9 yrs), adolescents (8–14.9 yrs), and adults (>15 yrs, male; after full sexual swelling, females). To control for age and satisfy small samples of individuals per age category, each individual was assigned to one of 11 age-classes. Assignation to age-class was determined by the individual's mean age between their start and end of observations, 1993-2006. Age-classes, together with sample sizes of females and males, respectively (total 37 females, 31 males), were: 0–1 yr (1,2), 1–2 yrs (5,4), 2–4 yrs (1,5), 4–6 yrs (3,2), 6–8 yrs (4,1), 8–10 yrs (1,2), 10–12 yrs (1,1), 12–14 yrs (1,1), 14–16 yrs (4,1), 16–20 yrs (2,1) and >20 yrs (14,11). Mean stick-carrying rates across individuals were higher for females than for males (Wilcoxon signed-rank T = 3; n = 10 age-classes, P = 0.017 (2-tailed)). No stick-carrying was observed for individuals in the 8–10 year age-class. Note that although the figure shows stick-carrying by individuals whose mid-point was in the adult range, no female carried a stick after becoming a mother.