cell fate, does STAT function in the cell cycle? It was previously shown that embryos lacking maternal and zygotic cdk4 function show defects in the BrdU labeling pattern specifically in endoreplicating tissues such as the midgut at state 14 (Meyer et al., 2002). Chen et al. show a similar BrdU labeling pattern in embryos lacking maternal and zygotic STAT. So whereas neither Cdk4 nor STAT is necessary for cell cycle progression in all dividing cells of the Drosophila embryo, the two proteins appear to serve similar functions in regulating progression through endoreplication cycles.

Future Directions
This study clearly demonstrates a new function for Cdk4 in regulating cell fate and pattern formation through STAT, and this role is apparently independent of effects on the cell cycle. Moreover, the study suggests a new mechanism for activating STAT. Many questions remain for future study, such as whether STAT and Cyclin-Cdk complexes bind to each other at physiological protein concentrations in the embryo. Another question is what signals control Cyclin D-Cdk4 expression and activation in this context. STAT protein levels have been previously reported to be dramatically lower in hop mutant embryos (Chen et al., 2002). Because Cyclin D is a known target of STAT in mammalian cells (Bromberg, 2001), one possibility is that activation of STAT via JAK leads to elevated expression or activity of Cyclin D and/or Cdk4, and this feeds back by activation of Cyclin E-Cdk2 to stabilize and activate STAT. However, further experiments are required to test this hypothesis. A third open question is whether the relationship between Cyclin-Cdk and STAT reported here is present in other cell types and organisms. Chen et al. investigated genetic interactions between these components in two other tissues, the developing eye and macrophage-like cells known as hemocytes. Although the relationship between JAK/STAT and Cyclin-Cdk in these tissues is not as straightforward to interpret, Chen et al. point out similarities between the effects on cell growth in these tissues and the mouse knockout phenotypes for Cyclin-Cdk and STAT. Finally, both of these pathways have been implicated in cancer, and it will be of interest to investigate whether crosstalk between Cyclin-Cdk and JAK/STAT contributes to tumorigenesis (Bromberg, 2001; Sherr and Roberts, 1999).

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Selected Reading

A Structural View of Integrin Activation and Signaling

Integrins connect the matrix to the cytoskeleton and propagate structural order between the two systems. A series of elegant structural papers now provides a compelling explanation of how integrins perform this basic function.

The integrins are a class of adhesion receptors that link the extracellular matrix to the cytoskeleton and cooperate with growth factor receptors to promote cell survival, cell cycle progression, and cell migration (Giancotti and Ruoslahti, 1999). The integrins consist of an α and a β subunit. Each subunit has a large extracellular portion, a single transmembrane (TM) segment, and a short cytoplasmic domain (with the exception of β4). The N-terminal domains of the α and β subunits associate to form the integrin headpiece, which contains the ligand binding site, whereas the C-terminal segments traverse the plasma membrane and mediate interaction with the cytoskeleton and with signaling proteins. Integrins can signal in both directions: matrix binding promotes association of the integrin with the actin cytoskeleton and activates biochemical signals inside the cell (signaling; Giancotti and Ruoslahti, 1999). Conversely, intracellular signals can induce the integrin to bind to its matrix ligand (activation; Liddington and Ginsberg, 2002).

Recent structural studies buttressed by ingenious mutations and rotary shadowing EM analyses have revealed that integrin signaling and activation are mediated by large conformational changes that are propagated from the integrin headpiece to the cytoplasmic domains and vice versa, respectively (Liddington and Ginsberg, 2002; Shi maoka et al., 2002). The integrins exist in two major allosteric conformations, inactive (low-affinity state) and active (high-affinity state). The low-affinity state, which appears to be the default state, is maintained by a weak interaction between the C-terminal portions of the transmembrane segments of the two subunits (handshake or clasp). Upon binding to the integrin’s headpiece, the matrix ligand induces conformational changes that are propagated along the integrin: the two legs, which are initially bent, undergo a “switch blade” movement, they straighten up, and the weak bond between the ends of the transmembrane segments of the α and β subunits is resolved. Release of this conformational restraint likely causes the α and β subunit cytoplasmic tails to move...
away from one another so that the cytoplasmic segment of the β subunit can interact with the cytoskeleton (see Figure). Conversely, the intracellular activators are thought to interact with either the α or the β subunit tail and induce the C-terminal ends of TM segments and the cytoplasmic domains to separate from each other. The ensuing switch blade-like straightening of the legs would cause further conformational changes that open the binding site on the headpiece, allowing the integrin to bind to its matrix ligand with sufficient affinity (see Figure). In other words, integrin activation and signaling are, at the molecular level, one and the same process.

What is the relationship between integrin activation and association with the cytoskeleton? Talin, best known for its ability to link integrins to the actin cytoskeleton, has recently emerged as an integrin activator (Calderwood et al., 1999). Thus, as the matrix ligand acts as an activator, this intracellular activator functions as an internal ligand as well as a cytoskeletal linker, reinforcing the theme of simplicity. Talin consists of the head domain (talin-H), which displays significant sequence homology to the FERM domain present in ERM (ezrin, radixin, and moesin) proteins, and a rod domain. An interaction between talin-H and the C-terminal portion of the rod masks the integrin binding site in the head. This autoinhibition is resolved upon binding of PIP2 to talin or proteolytic separation of the head from the rest of the molecule, extending further the analogy to ERM proteins (Martel et al., 2001; Calderwood et al., 1999; see Figure). The FERM domain consists of three subdomains, F1–F3, arranged as leaves in a clover. F3 resembles a PTB domain, which can bind to NpxY motifs in a phosphorylation-dependent or -independent fashion. Notably, the interaction of talin with integrins is disrupted by mutations in the NpxY motif in the β subunit tail, implying a resemblance with known PTB-peptide interactions (Calderwood et al., 1999).

Two recent studies have provided a particularly vivid view of the interaction of the cytoplasmic domains of the integrin αIIbβ3 with talin. Vinogradova et al. (2002) used NMR spectroscopy to provide evidence that a peptide that includes the C-terminal end of the TM segment and the cytoplasmic tail of the α subunit (K989–E1008) interacts with a peptide that encompasses the same region of the β subunit (K716–T762). Spectral perturbations were observed in the N-terminal (K989–N996) and the cytoplasmic domains to separate from each other. The ensuing switch blade-like straightening of the legs would cause further conformational changes that open the binding site on the headpiece, allowing the integrin to bind to its matrix ligand with sufficient affinity (see Figure). In other words, integrin activation and signaling are, at the molecular level, one and the same process.
F3 of talin-H—which are sufficient to activate integrins—alone and in complex with a β3 peptide encompassing the NPLY motif. The complex was obtained through crystallization of chimeras containing β3 residues W739–E749 or W739–A750 fused to the talin F2 + F3 fragment and was validated by mutagenesis. There are several notable features in the new structure. Like other F3 subdomains, talin F3 consists of two antiparallel β sheets enclosing a hydrophobic core. A single C-terminal α helix encloses one edge of the β sandwich inserting between strands β5 and β1. The overall fold is most similar to that of the PTB domain of IRS-1. The β3 peptide establishes interactions with a predominantly hydrophobic surface delimited by the β5 strand, the C-terminal part of helix 5, and the β4–β5 loop of talin F3. The segment of the β3 peptide upstream of the NPLY motif forms a β strand that increases the β sheet formed by β5, β6, and β7 of F3. As previously predicted, the 744 NPLY motif forms a reverse turn and N744 makes hydrogen bonds with the turn as well as with T354 and I356 of talin.

There are two key interactions (see box in Figure). The first involves the side chain of Y747, which fills an acidic and hydrophobic pocket delimited by the ends of strands β5 and β6 of F3 and by L748 in the turn. By contrast, the PTB domains that bind to phosphorylated NpxY motifs accommodate the phosphotyrosine in a very basic pocket. These observations suggest that phosphorylation of the NpxY motif in the β subunit tail may reverse, rather than promote, integrin binding, in accordance with prior observations in cells transformed by oncogenic tyrosine kinases. The second key interaction is established by W739 at position -8 with respect to the tyrosine. Its side chain occupies a pocket formed by R358, A360, and Y377 of talin F3. This interaction appears to be well conserved, as most integrin β subunits possess an aromatic residue in the same position.

Together, these observations indicate that the talin F3 subdomain establishes a PTB-peptide interaction with a central portion of the integrin β subunit tail that encompasses the NpxY motif. How does this result in integrin activation? It is conceivable that talin binds first to the available high-affinity binding site in the central region of the β tail and subsequently to a lower affinity binding site in the helical portion at the end of the transmembrane segment, causing separation of the two tails. It is also possible that allosteric mechanisms contribute to perturbing the “clasp” after talin binds to its principal binding site in the β tail. Future studies will undoubtedly resolve this outstanding issue.

How does the new work relate to the signaling function of integrins? Although it is too early to tell, there are hints suggesting that we may soon have a more comprehensive view of the basic mechanism of integrin signaling. Li et al. (2001) have demonstrated that the transmembrane segments of αIIb and β3 form SDS-resistant homodimers and homotrimers, respectively. It is interesting to note that the TM segments of most integrin α subunits contain a GxxxG motif that has been shown to mediate association of transmembrane helices (Russ and Engelman, 2000). Similarly, many β subunit TM segments contain a small aliphatic side-chain-cluster motif that has been implicated in multimerization of growth factor receptors (Bormann and Engelman, 1992). It is conceivable that, upon separation of the tails, the observed homooligomeric associations of α and β subunit TM segments promote integrin aggregation on the plasma membrane (see Figure). This model readily explains the tight coupling between integrin affinity and avidity of binding to the matrix. If validated, it may also provide a framework on which to build a biophysical understanding of integrin signaling.

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