Survival of the weakest: signaling aided by endosomes

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The tyrosine kinase receptor c-Met plays a key role in cell proliferation, morphogenesis, and motility in response to hepatocyte growth factor. C-Met is often altered in cancer and is a major target for therapeutic intervention. Despite knowing a great deal of the molecular machinery downstream of this receptor tyrosine kinase, the spatiotemporal regulation of c-Met signaling still remains elusive. In this issue of the Journal of Cell Biology, Kermorgant and Parker (Kermorgant, S. and P.J. Parker. 2008. *J. Cell Biol.* 182:855–863) provide evidence for a model in which the c-Met–activated STAT3 signal is mediated by endosomal trafficking. This study elegantly highlights how weak signals can be effectively transmitted to the nucleus by exploiting endosomal compartments, raising important mechanistic implications for the signaling research community.

Hepatocyte growth factor (HGF), also known as scatter factor, binds to its receptor, the c-Met tyrosine kinase, to induce cell proliferation, migration, morphogenesis, and survival. These varied responses are the result of diverse signaling pathways, including those activating ERK1/2, STAT3, Rac, and Akt (Birchmeier et al., 2003). In many cancers, c-Met is overexpressed, activated, amplified, and/or mutated and, thus, it is a major proto-oncogene heavily targeted for therapy (for review see Comoglio et al., 2008). How exactly the different pathways downstream of HGF/c-Met are specifically regulated and coordinated still remains elusive.

One downstream signaling molecule of c-Met is the multifunctional transcription factor STAT3 (signal transducer and activator of transcription). Under basal conditions, unphosphorylated STAT3 constitutively cycles between the cytoplasm and the nucleus (Liu et al., 2005). Binding of HGF to c-Met results in recruitment of STAT3 to c-Met, the phosphorylation of STAT3, and STAT3 nuclear accumulation (Boccaccio et al., 1998). The first suggestion that endocytosis might be important for STAT3 signaling came from experiments showing that EGF stimulation of cells resulted in STAT3 localization to endosomes and endocytosis was important for STAT3-dependent transcription (Bild et al., 2002). Endocytosis was later demonstrated to be involved in some HGF-mediated signaling events (Hammond et al., 2003; Kermorgant et al., 2003, 2004) including HGF-induced Rac trafficking in cell migration (Palamidessi et al., 2008). The questions of why c-Met–dependent STAT3 activation should require transport to endosomes instead of free diffusion through the cytoplasm and of how universal this mode of STAT3 signaling is remain a mystery.

In this issue of the Journal of Cell Biology, Kermorgant and Parker (see p. 855) provide a possible answer to these questions by demonstrating an unexpected relationship between the strength of the signaling response (e.g., activation by phosphorylation) and the trafficking of the receptor and downstream signaling components. The authors compared the activation of ERK1/2 and STAT3 with the intracellular trafficking of c-Met in response to HGF stimulation. The authors found that HGF elicits a potent activation of ERK1/2 that requires internalization of c-Met into endosomes but does not require its trafficking to a perinuclear compartment (Fig. 1). In contrast, STAT3 activation is comparatively "weak" and requires the localization of active c-Met to the perinuclear region via a microtubule and PKCα-dependent process (Fig. 1). Interestingly, STAT3 activation via the cytokine Oncostatin M produces a "stronger" signal that occurs independently of microtubules and PKCα (Fig. 1). This result demonstrates that the trafficking of STAT3 to endosomes is not an absolute requirement for activity but rather depends on the "signal strength" elicited by the growth factor–receptor complex. The involvement of the endocytic pathway in STAT3 signaling was also recently shown for IL-6 and, if it follows the trend established here, then one would predict the IL-6–mediated STAT3 signal also to be weak (Shah et al., 2006).

The findings presented in this study pose several interesting problems for the entire field of receptor-mediated signaling and membrane trafficking. Increasing evidence has been accumulating in favor of the "signaling endosome" hypothesis, whereby signals are sustained or generated on endosomes and compartmentalization is exploited to generate quantitative and qualitative

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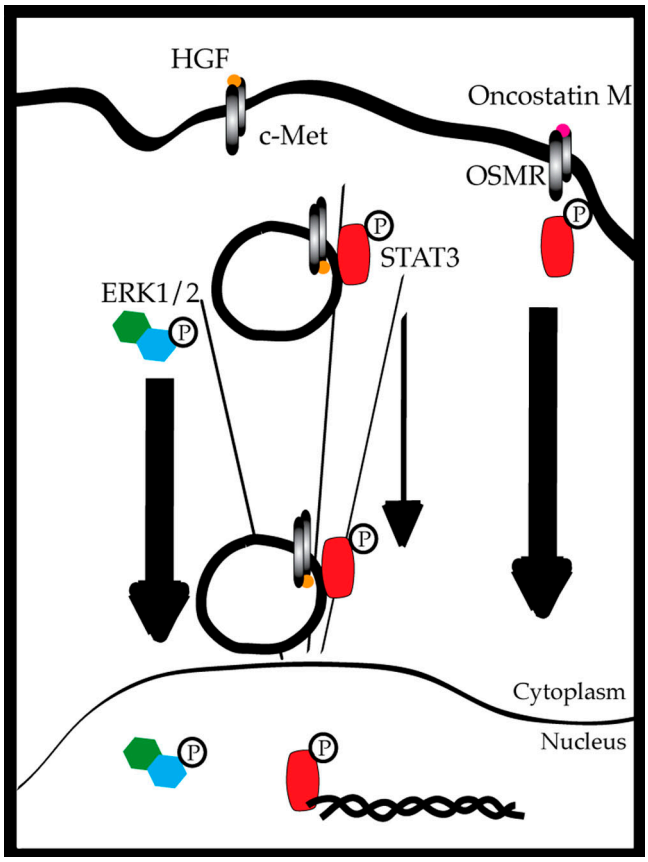

Figure 1. **Survival of the weakest.** Both ERK1/2 and STAT3 signaling require the endocytosis of the HGF-bound c-Met receptor. The signal strength differs between these two signaling proteins and this leads to disparate pathways to the nucleus. The strong ERK1/2 signal proceeds via cytosolic diffusion, whereas the weaker STAT3 signal requires a microtubule-dependent perinuclear localization of c-Met. In comparison, a strong STAT3 signal mediated by Oncostatin M proceeds similarly to ERK1/2. OSMR, Oncostatin M receptor.

differences in signals. Some of the fundamental questions follow. Which objective criteria define signal strength? In the current study, only a systematic quantitative functional analysis coupled to mathematical modeling can provide a precise assessment of signal strength for each signaling system. Furthermore, which molecular features of the signaling machinery determine the signal strength and to what extent are endosomal compartments involved in this regulation? Signal strength can vary in a signaling cascade and quantitative differences may be exploited to generate different outcomes (Santos et al., 2007). The current study raises the question of why STAT3 traffics via endosomes. STAT3 may need to dynamically associate with activated c-Met, as suggested by Kermorgant and Parker (see p. 855), perhaps engaging with endosomal signaling complexes yet to be identified (as in the case of the p14–MP1–MAPK complex on late endosomes (Wunderlich et al., 2001). There may be a conformational change that occurs upon binding to either the c-Met receptor in an endosomal compartment or other endosomal proteins. Another potential explanation is that the weak STAT3 signal may "survive" on endosomes where it may be protected from premature inactivation by the highly enriched cytoplasmic milieu of phosphatases (Birtwistle et al., 2007).

Another question is which type of endosomal compartment is involved in c-Met–dependent STAT3 signaling? The identity of the perinuclear endosomal compartments accessed by c-Met over time has not yet been well characterized. Signaling could be elicited from recycling endosomes or late endocytic compartments, both of which are positioned mainly in the perinuclear region of the cell. This question is important in view of recent data implicating a newly identified endosomal compartment (APPL-positive endosomes) with an unexpected role in signal specificity (Miaczynska et al., 2004; Schenck et al., 2008). As shown for several receptors (Lin et al., 2006; Mao et al., 2006; Erdmann et al., 2007), c-Met (at least a fraction of receptor) could also enter and signal from APPL endosomes. It would be informative to determine if there is also a STAT3 APPL-positive endosomal population and, if so, whether there is a functional specificity to this endosomal STAT3 signal.

Interestingly, the transport of transcription factors via endosomes to a perinuclear location and the concomitant evidence of endosomal proteins found in the nucleus suggest that there is a widespread role for endocytic proteins in transcriptional regulation (Pilecka et al., 2007). The authors of the present study did not address the transcriptional activity of STAT3 but, instead, equated nuclear presence to transcriptional activity. Are there, in fact, functional differences between the transcriptional profiles of soluble cytosolic STAT3 versus STAT3 activated on endosomes? STAT3 has recently been shown to have an additional role in the cytoplasm. It can bind to the microtubule binding protein stathmin and antagonize its microtubule destabilizing activity leading to a reorganization of the microtubule network (Ng et al., 2006). Clearly, we need to explore further the role of signaling molecules as trafficking regulators, as signal transduction is known to modulate membrane and cytoskeleton dynamics.

The functional outcome of c-Met activation is a fine balance between signaling pathways using diffusion and those requiring endosomal trafficking. As presented in this issue, HGF binding to c-Met results in the compartmentalization of activated STAT3 to endosomes thus allowing a weak signal to reach the nucleus. These studies have been conducted in HeLa cells as a model system. Next, it will be important to validate the conclusions of this study in model organisms, where mechanistic differences can be highlighted under physiological conditions.

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