

Figure 1. The Genetic Events in Mice and Human Cells Necessary for Tumorigenesis.

See text for details.

there was a selection of cells capable of overcoming a deficiency in complete Ras signaling in these two cases. Hence, it will be interesting to determine whether the missing Ras effector pathway is activated in cells of these tumors. However, if activating only a subset of Ras effectors in a cell type specific fashion can indeed induce tumorigenic growth, then Ras-mediated tumorigenesis just got a whole lot messier. This begs the question of whether there even is a common effector that could be universally targeted in Ras<sup>12V</sup>-expressing tumors. In this regard, it is worth noting that the RalGEF pathway is essential for Ras-mediated transformation or tumorigenesis in a variety of human cell types (Hamad et al., 2002), and in the two cases in which tumors were induced by pairs of Ras effector mutants in the Weinberg

## Arginine Methylation Regulates the Cytokine Response

The recent identification of the NFAT-interacting protein NIP45 as a methylated protein by Mowen et al. (2004) marks a new role for protein arginine methylation in lymphocyte signaling and cytokine gene activation.

Protein arginine methylation is a post-translational modification that adds methyl groups to the nitrogens of arginines (Gary and Clarke, 1998). Although the modification is well known, the functional consequences of arginine methylation as well as its regulation remains largely unknown. The cloning of the protein arginine methyltransferase (PRMTs) and the development of methylarginine-specific antibodies have generated re-

study, the mutant that activates RalGEFs was expressed in both cases (Rangarajan et al., 2004).

In summary, the Weinberg lab has been at the forefront of distilling cancer down to a distinct set of changes. By changing one parameter at a time (cell type, organism, specific genes) in this controlled system, they make the kind of direct comparisons in transformation or tumorigenesis previously not possible—opening the doors to testing whether other transforming events elucidated in mice function in a similar manner in human cells.

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newed interest in this modification in the last decade. In the past, arginine methylation was mainly observed on abundant proteins such as RNA binding proteins and histones, but a proteomic analysis for methylated proteins revealed other proteins implicated in a variety of cellular processes (Boisvert et al., 2003), demonstrating that arginine methylation may be participating in many cellular processes. However, a role for arginine methylation in T lymphocytes has resisted elucidation. In this issue of *Molecular Cell*, Mowen et al. (2004) provide the first example that arginine methylation promotes cytokine gene expression by regulating the activity of the NFAT transcription factors. The association of PRMT1 within the NFAT complex also highlights the coactivator function of PRMT1 in cytokine gene expression.

T cell receptor (TCR) engagement triggers a cascade of post-translational events leading to increased cytokine gene expression. The production of cytokines, a family of ligands, contributes to the environment that

dictates the fate of T helper precursors into different cell lineages. Phosphorylation and ubiquitylation are modifications known to regulate cytokine gene expression. The ensuing report by Mowen et al. (2004) now introduces arginine methylation in T cell receptor signaling and the cytokine response. The authors demonstrate that the NFAT coactivator NIP45 is arginine methylated by PRMT1. They show that the NFAT/NIP45 interaction requires arginine methylation for cytokine gene activation. It is known that secreted cytokines through an autocrine/paracrine loop bind their receptors and subsequently induce STAT-dependent gene activation. Previous studies by Mowen and colleagues and others demonstrated that arginine methylation of STAT proteins is required for cytokine receptor signaling (Chen et al., 2004; Mowen et al., 2001). Thus arginine methylation by PRMT1 regulates the cytokine response at two levels: (1) the initial response induced by NFAT transcription factors, and (2) the secondary cytokine signaling cascade induced by the STAT proteins (Figure 1). Both examples demonstrate that arginine methylation induces positive roles in cytokine gene activation and signaling.

Arginine methyltransferases are known to be abundant and in most cases, arginine methylation is constitutive. The question therefore is how is the methylation of NIP45 regulated? And how are the PRMTs regulated? Mowen et al. (2004) show that both PRMT1 and CARM1 protein expression are induced following lymphocyte activation. The methylation status of NIP45 was constitutive in lymphocytes and this raises the question as to whether the increase in methyltransferase expression correlates with an increase in global arginine methylation. Alternatively, the increased methyltransferase expression observed may only result in local increases in protein methylation such as cytokine gene promoters. The increased CARM1 expression following T cell signaling suggests that CARM1 may have a regulated role in lymphocytes. These observations are consistent with CARM1 null mice that display a differentiation defect in thymocyte progenitors (Kim et al., 2004). The increased PRMT1 and CARM1 expression in lymphocytes suggest that these methyltransferases may be functioning together with other coactivators, as recently described for the p53 promoter (An et al., 2004). In the case of PRMT1, NIP45 is the protein that is most likely recruiting it, perhaps in an analogous manner as was shown with YY1 (Rezai-Zadeh et al., 2003).

What other events of T cell signaling are mediated and regulated by arginine methylation? Mowen et al. (2004) provide one example with NIP45 and NFAT. The characterization of other methyltransferases and their substrates involved in TCR signaling should provide a better understanding of the role of PRMTs in the cytokine response. In a screen to identify methylated proteins, the interleukin enhancer binding factors, ILF1 and ILF2 were identified (Boisvert et al., 2003). These cytokine signaling factors are regulators of RNA metabolism and they may stabilize mRNAs encoding cytokines and PRMTs in a methylation-dependent manner.

How is the interaction between NIP45 and NFAT regulated? Hypomethylated NIP45 and N-terminal NIP45 deletion mutants that cannot be methylated by PRMT1 have reduced association with NFAT and failed to acti-

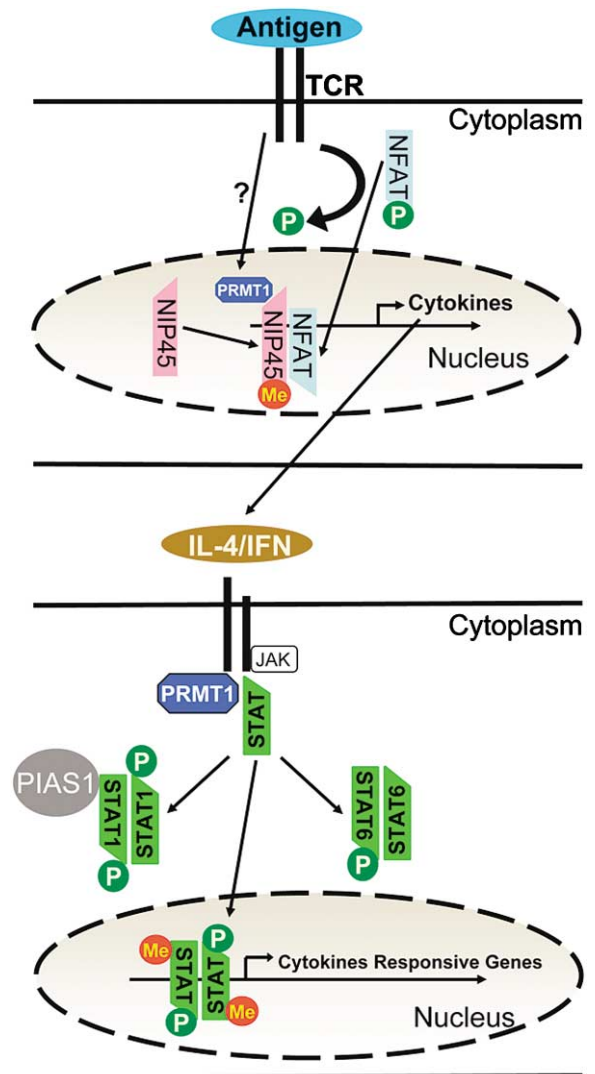


Figure 1. Arginine Methylation Is Involved in Two Different Steps of the Cytokine Response

Activation of the TCR induces dephosphorylation of NFAT transcription factors that then translocate to the nucleus, where they interact with methylated NIP45 to activate transcription of cytokine genes. Engagement of cytokine receptors then activates STATs of which only methylated STAT proteins translocated to the nucleus and activate transcription of cytokine responsive genes.

vate the cytokine response. It will be of particular interest to characterize how arginine methylation influences the interaction between NIP45 and NFAT: whether arginine methylation regulates the localization and the conformation of the proteins or whether the interaction is direct and methylarginine dependent. Protein arginine methylation can selectively modulate certain protein-protein interactions (McBride and Silver, 2001); however, the determinants involved in these interactions have not been elucidated and a methylarginine binding protein module has remained elusive.

The regulation of arginine methylation implies that there may be a mechanism to downregulate this modification and contrary to phosphorylation, arginine methylation has always been considered irreversible, or at least

a modification that is not very labile. Thus, arginine methylation may have evolved as a modification that is more sustainable than phosphorylation. Arginine methylation may provide diversity in cytokine signaling cascades for prolonged responses.

Overexpression of cytokines leads to several chronic diseases such as allergies, asthma, autoimmunity, and cancer. The finding that arginine methylation plays an important role in the control of expression of cytokines, as well as in the response to cytokines activation might provide new therapeutic targets for intervention in these diseases.

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## The End in Sight

**The HP1 heterochromatin protein has become an archetypical example of a chromatin protein recruited by binding a specific histone modification. Surprisingly, recent work in *Drosophila* reveals that the essential function of HP1 is accomplished by another mode of binding.**

In 1940 Muller concluded from irradiation experiments with *Drosophila* that chromosome ends are special elements, which he termed telomeres. Telomeres were defined by their difference from induced broken ends, which rapidly recombine or fuse with other breaks. Muller envisaged that telomeres act as “caps,” and end-to-end fusion is now diagnostic of defective telomere capping. Structurally, telomeres are often associated with sub-telomeric blocks of heterochromatin, while the extreme end itself is heterogeneous in length and consists of short, G- and T-rich (GT) repeats. A second property of telomeres was inferred from the primer requirement for lagging strand DNA synthesis. This “end-replication problem” is solved in most eukaryotes by the ribonucleoprotein telomerase, which adds GT repeats to the chromosome end. A variety of proteins specifically bind the GT tracts and are involved in preventing end-to-end fusion. Thus, telomerase-catalyzed extension accomplishes both maintenance of chromosome length and recruitment of capping proteins.

Perplexingly, telomerase is found in most organisms, but *Drosophila* is different. Fly chromosomes have no terminal GT repeats; instead, telomeric sequences are often the HeT and the TART retroposons. *Drosophila* appears to have solved the end-replication problem by harnessing these retroposons: at a low frequency HeT and TART jump onto the end of the chromosome, provid-

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ing a buffer of expendable sequences. But even these sequences prove to be non-essential for telomere capping: broken chromosomes can be isolated, and these “terminal deficiency” chromosomes end in unique genomic sequence. The broken chromosomes slowly grow shorter, but do not fuse with other ends. The variety of sequences at the ends of *Drosophila* chromosomes precludes sequence-specific binding as a mechanism to recruit capping proteins. Instead, there must be other ways to recognize the natural chromosome end.

In a recent issue of *Molecular Cell*, Perrini et al. clarify the relationship of *Drosophila* telomeres to those in other organisms by analyzing the role of the heterochromatin protein HP1. Attention has focused on the interaction between HP1 and lysine-9-methylated histone H3 (H3K9-me) as a way of recruiting this repressor to specific chromatin sites. However, a dizzying array of observations in *Drosophila* do not fit this paradigm. For example, elimination of the SuVar3-9 methyl-transferase which catalyzes H3K9 methylation is viable (Schotta et al. 2002), as is elimination of the RNAi machinery that directs H3K9 methylation (Pal-Bhadra et al., 2004). In contrast, HP1 itself is essential, implying that HP1 has methylation-independent functions. Perrini et al. resolve this issue by detailing a separate mode of binding for HP1 to the chromosome end. The authors use a specific DNA adduct as a cross-linking reagent to demonstrate that HP1 directly binds DNA, both in vivo and in vitro. HP1 binding to the end of the chromosome is independent of the RNAi-directed system that specifies histone methylation. Furthermore, binding is not due to the repetitive HeT and TART elements, because HP1 can be cross-linked to unique sequence on the ends of terminal deficiency chromosomes. This sequence-independent DNA binding property of HP1 is suitable for recognizing the ends of *Drosophila* chromosomes.

So is HP1 the telomere cap? Telomeres are thought to resemble double-strand breaks that are prevented from initiating DNA damage responses and repair. There