LKB1 and AMPK Family Signaling: The Intimate Link Between Cell Polarity and Energy Metabolism

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Jansen M, ten Klooster JP, Offerhaus GJ, Clevers H. LKB1 and AMPK Family Signaling: The Intimate Link Between Cell Polarity and Energy Metabolism. Physiol Rev 89: 777–798, 2009; doi:10.1152/physrev.00026.2008.—Research on the LKB1 tumor suppressor protein mutated in cancer-prone Peutz-Jeghers patients has continued at a feverish pace following exciting developments linking energy metabolism and cancer development. This review summarizes the current state of research on the LKB1 tumor suppressor. The weight of the evidence currently indicates an evolutionary conserved role for the protein in the regulation of various aspects of cellular polarity and energy metabolism. We focus on studies examining the concept that both cellular polarity and energy metabolism are regulated through the conserved LKB1-AMPK signal transduction pathway. Recent studies from a variety of model organisms have given new insight into the mechanism of polyp development and cancer formation in Peutz-Jeghers patients and the role of LKB1 mutation in sporadic tumorigenesis. Conditional LKB1 mouse models have outlined a tissue-dependent context for pathway activation and suggest that LKB1 may affect different AMPK isoforms independently. Elucidation of the molecular mechanism responsible for Peutz-Jeghers syndrome will undoubtedly reveal important insight into cancer development in the larger population.
I. INTRODUCTION

In recent years, research on the LKB1 tumor suppressor protein has intensified at a staggering pace. Historically, interest in the protein was first aroused in 1998 after it was found that germline inactivating mutations cause the rare cancer-prone Peutz-Jeghers syndrome (PJS). Although initial studies appeared to suggest a limited role for the protein in sporadic malignant tumor progression, it has now become evident that LKB1 is frequently targeted for inactivation during sporadic lung cancer transformation. In addition, studies now suggest a role for LKB1 in metabolic regulation through the well-known AMP-activated protein kinase (AMPK) module. For this reason, LKB1 has now been implicated in a vast range of clinical conditions ranging from diabetes mellitus to pulmonary cancer. As these conditions represent primary causes of morbidity in the Western world, LKB1 has become a formidable target for drug therapy. In keeping with this range of physiological processes and associated clinical disorders, LKB1 is now linked to a broad plethora of downstream targets. This review aims to provide an overview of the current state of research on the LKB1 tumor suppressor protein.

II. PEUTZ-JEGHERS SYNDROME

A. Historical Account

Peutz-Jeghers is a rare cancer predisposition syndrome characterized by the development of gastrointestinal polyps and mucocutaneous pigmentation abnormalities. The syndrome was first described in 1921 by the Dutch physician Dr Johannes Peutz working in The Hague (23, 80, 93). He documented the case of a 15-yr-old boy that had been surgically treated for a small bowel intussusception. On rectoscopy, Dr. Peutz noted multiple rectal polyps in this patient. Further investigation in siblings led to the identification of similar rectal polyps. All siblings affected by gastrointestinal polyps similarly suffered from nasal polyps and perioral hyperpigmentation. The syndrome was further characterized by Dr. Harold Jeghers in 1949, who recognized that “a single pleiotropic gene was responsible for both characteristics, the polyps and the spots.” PJS is an autosomal dominant cancer-prone gastrointestinal polyposis disorder. The disorder presents in 1 per 50,000 to 1 per 200,000 newborns and is caused by germline mutation in the \( LKB1 \) gene. Due to the rarity of the syndrome, clinical accounts of pedigrees afflicted by the syndrome are rare, and accurate documentation of the increased cancer-risk is therefore complex. Based on large cohort studies, patients are estimated to display an 18-fold increased cancer risk over the normal population (43).

B. Clinical Characteristics

The first sign of PJS is commonly a distinctive pigmentation observed around the lips, oral mucosa, genitalia, or palmar surfaces appearing early in childhood (Fig. 1) (60, 80). However, the polyps constitute the main clinical symptom. These may develop throughout the gastrointestinal tract, grow to large sizes, and cause occlusions, pain, and gastrointestinal bleeding with anemia. Polyps in PJS appear to predominate in the small intestine. A recent study involving repeated gastroscopies in PJS patients showed the presence of hundreds of smaller polyps in the stomach of all analyzed patients (123). These small, nascent PJS polyps lacked the prominent smooth muscle stalk typical of larger PJS polyps, but did show signs of a hyperproliferative epithelium, and were classified as hyperplastic polyps, accordingly. These observations suggest that counting polyps limited to those that come to clinical attention might misrepresent actual numbers and...
distribution of polyps. Polyps in PJS have also been described at locations outside of the gastrointestinal tract such as the nasopharynx, gallbladder, trachea, and urogenital tract.

The large gastrointestinal polyps typically encountered in PJS, which may develop from the smaller hyperplastic lesions, are pedunculated polyps with nondysplastic overlying epithelium. A characteristic feature of mature PJS polyps is the prominent core of arborizing smooth muscle, which extends into the head of the polyp. Smooth muscle proliferation appears to accompany epithelial hyperproliferation during early stages of polyp development (54). It is important to realize that smooth muscle proliferation is not a histopathological feature specific for the PJS polyp. For example, PJS-like smooth muscle proliferation can also be observed in conventional sporadic adenomatous polyps. In this instance, the smooth muscle proliferation develops secondary to mechanical insults (due to intestinal peristalsis) during adenomatous polyp growth (54). Furthermore, other conditions unrelated to PJS such as mucosal prolapse syndrome may present with histological features similar to those observed in PJS polyps as well (54, 107). Thus it is unclear whether the observed smooth muscle proliferation is causally involved in polyp initiation or, alternatively, whether it develops as an epiphenomenon to accommodate for epithelial hyperproliferation.

The potential for malignant derailment of PJS polyps and, specifically, whether cancer in PJS patients arises from the nondysplastic epithelium covering PJS polyps remains a hotly debated subject. This debate centers on the key question of how malignant derailment in PJS patients models malignant tumor progression in sporadic patients, i.e., cancer patients not affected by PJS. Analogous to the lessons learned from the stepwise tumor progression that occurs in familial adenomatosis polyposis (FAP) patients, it is hoped that neoplastic transformation in PJS patients similarly models events during malignant tumor progression, which can then be extrapolated to the public at large. A firm understanding of the histopathological mode of cancer initiation in PJS patients is critical to begin to unravel the role of LKB1, and the consequences of its loss, during sporadic tumor progression. Because of their marked presentation, the gastrointestinal polyps in PJS constitute a prime suspect, but do they really harbor an underlying risk for malignant transformation in spite of their benign appearance? Taking the above-mentioned case of a conventional adenomatous polyp displaying a prominent PJS-like core of arborizing smooth muscle as an example, it would on histopathological grounds alone not be possible to discern whether one is observing an adenomatous polyp that secondarily acquired a PJS-like smooth muscle stalk or, alternatively, a PJS polyp that has undergone adenomatous transformation (Fig. 2). Historically, case reports have described dysplastic adenomatous transformation occurring in a supposedly preexistent “hamartomatous” PJS polyp (83). However, dysplasia occurring in classical PJS polyps appears to be a very uncommon phenomenon. In series from the renowned familial polyposis registry at St Mark’s hospital, a review of 491 polyps showed no evidence of dysplasia in any of them (106). Moreover, follow-up over

![Fig. 2. PJS-like smooth muscle proliferation in a conventional non-PJS adenomatous polyp. A: gross macroscopy of a pedunculated colonic polyp (indicated by * in A) in a 67-yr-old patient not affected by PJS. A right hemicolectomy was performed following the diagnosis of colon cancer in the ascending colon in this patient; the polyp shown here was found incidentally in the cecum. B: low-power photomicrograph of the polyp shown in A. The polyp has a tubulovillous architecture characterized by long fingerlike glands emanating from the stalk of the polyp. Note the slender extensions of the smooth muscle core into the tips of the villous projections (indicated by * in C). This arborizing core of smooth muscle resembles the histology shown in Fig. 1B for the polyp removed from a PJS patient. C: unlike PJS polyps, which are characterized by a nondysplastic hyperproliferative epithelial lining, this polyp has a dysplastic epithelial covering classified as low-grade dysplasia. The boxed area in B is shown at higher power in C. Polyps removed from PJS patients have been reported to contain dysplastic foci, which have been taken as evidence of malignant potential of PJS polyps in spite of their benign histological appearance (see sect. II B on models of polyp development in PJS). However, conventional preneoplastic adenomatous polyps arising in PJS patients may similarly have features such as a prominent smooth muscle core along the stepwise progression towards an invasive carcinoma, as shown in this example. PJS polyps as shown in Fig. 1B may therefore be an epiphenomenon to the malignant predisposition in PJS and not preneoplastic per se.](http://physrev.physiology.org/)

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45 years of 48 patients at the Mayo Clinic failed to reveal dysplastic change in any polyp as well (75). More recently, we have proposed that these historical accounts of adenomatous transformation occurring in PJS polyps might be explained by a blurring of the order of events; that is, these lesions might likely have arisen due to a conventional adenoma arising in a PJS patient that secondarily developed a PJS-like smooth muscle stalk, akin to the situation encountered in sporadic patients, as described above (54). This is addressed in greater detail in section vB on models of polyp development.

In view of the fact that the malignant potential of PJS polyps, and by inference the focus of cancer initiation in PJS patients, remains unclear, proper classification of PJS polyps is a relevant issue. Traditionally, mature PJS polyps have been classified as hamartomas, which signifies a nondysplastic overgrowth of all tissue layers native to the site of origin of the lesion in equal proportion. It is important to recall, however, that the “hamartoma” designation for PJS polyps is not based on a pathophysiological rationale at present. The hamartoma classification may therefore be misleading (and may ultimately prove to be a misnomer), since the actual etiological mechanism of polyp development remains unclear. This consideration is also relevant with regard to a number of studies that have appeared in literature mechanistically linking the so-called hamartoma syndromes PJS, tuberous sclerosis, and Cowden’s disease (24). Irrespective of whether a definitive molecular link can be established in future (see sect. viD on the mTOR module), from a clinical point of view it appears artificial to lump these syndromes together since they share little more clinical overlap than the hamartoma designation per se. Further research examining the histological architecture of PJS polyps in closer detail is therefore warranted. For example, even though the hamartoma designation mandates that the epithelial covering of PJS polyps would display all lines of differentiation normally observed at the site of origin of a PJS polyp in a normal ratio, earlier studies have pointed out that with regard to the epithelial covering of PJS polyps, some differentiated epithelial lineages may be lost at the expense of other lineages or in favor of a more immature phenotype altogether (46). The net shift that occurs towards an immature phenotype is not to be interpreted as a sign of premalignant potential. For these reasons, and to avoid clouding the debate, it appears prudent to refer to the gastrointestinal lesions in PJS as “polyps” or “PJS polyps” rather than hamartomas, since the latter would imply an etiological understanding that is currently not justified. Most importantly, because the malignant potential of PJS polyps, i.e., those polyps demonstrating a prominent smooth muscle core with normal nondysplastic overlying epithelium, remains uncertain, PJS polyps should be clearly separated from other (pre)neoplastic lesions occurring in Lkb1 mouse models (50). Accurate classification of PJS polyps thus awaits elucidation of the sequence of events during PJS polyp initiation and the pathophysiological mechanism responsible for PJS polyp development.

C. Cancer Risk and Surveillance

Mechanical problems due to polyp development in PJS dominate the first two decades of life, but with advancing age intestinal and extraintestinal cancer development becomes a major clinical concern. The cancer spectrum consists mostly of gastrointestinal cancers (colorectal and pancreatic) (43, 74). However, an increased risk for extraintestinal cancers has also been noted, and the risk of breast cancer in female PJS patients is comparable to that associated with either BRCA1 or BRCA2 mutations (43). Thus, compared with other cancer-prone conditions, PJS patients display an unusually wide tumor spectrum. In addition, PJS patients are at increased risk for distinctive benign tumors of the genital tract such as sex cord tumors with annular tubules (SCTATs) in affected female patients. SCTATs in PJS are often bilateral, multifocal, and benign and have been described as characteristic for the condition (135). SCTATs in patients not affected by PJS are typically unilateral and often display a malignant clinical course, in contrast to its benign character in PJS. Even though the absolute risk for the development of these gonadal tumors is only modestly increased, their occurrence is noteworthy as it may provide an important clue to the underlying mechanism of PJS polyp development and neoplastic transformation in PJS (see below).

The range of organs affected in PJS translates into a cumbersome follow-up strategy, and current recommendations include biannual upper endoscopy with polypectomy, colonoscopy with polypectomy, and small bowel X-ray series (Table 1) (36). However, a rational basis for surveillance strategies in these patients is sorely lacking. A recent analysis of the psychosocial impact of PJS in affected patients has shown that a diagnosis of PJS affects

<table>
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<th>Screened Organ</th>
<th>Starting Age, yr</th>
<th>Interval, yr</th>
<th>Tests</th>
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<tr>
<td>Colon</td>
<td>25</td>
<td>2</td>
<td>Colonoscopy</td>
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<tr>
<td>Proximal GI, small intestine</td>
<td>10</td>
<td>2</td>
<td>Endoscopy, small bowel barium X-ray series</td>
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<td>Pancreas</td>
<td>30</td>
<td>1–2</td>
<td>Endoscopic ultrasound</td>
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<td>Breast</td>
<td>20</td>
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GI, gastrointestinal tract. Data from Giardiello and Trimbath (36).
many important life decisions in these patients even though physically patients do not feel impacted compared with the general population (131).

III. CLONING OF THE LKB1 TUMOR SUPPRESSOR GENE

The LKB1 gene was cloned after comparative genomic hybridization (CGH) analysis on PJS polyps, which reportedly showed loss at chromosome 19p13 in a number of polyps removed from patients (46). Subsequent studies involving linkage analysis in PJS pedigrees confirmed linkage to this region. This inspired a commendable search for mutations in genes located in this genomic region, which allowed for the identification of mutations in the LKB1 gene (45, 55). Initial studies suggested locus heterogeneity, since LKB1 mutations were not detected in a significant number of cases. However, with the advent of techniques to identify large genomic deletions, it became clear that germline LKB1 inactivation is the central culprit (5, 29, 44). Indeed, sequencing analysis of interacting partners of LKB1 or potential downstream effectors showed, perhaps surprisingly, that Lkb1 inactivation is the central event in the human stomach (59, 123). Recent data show that Lkb1 is haploinsufficient for suppression of polyp development (9, 57, 85, 92, 127).

The identification of the LKB1 tumor suppressor gene afflicted in PJS patients prompted the analysis of LKB1 sequence changes in sporadic patients. Initial studies showed, perhaps surprisingly, that LKB1 was only infrequently biallelically targeted in sporadic tumorigenesis (e.g., <5% of sporadic colorectal carcinomas show biallelic inactivation) (101, 122). In contrast, recent research now shows that LKB1 is frequently targeted for inactivation in sporadic pulmonary cancers, in particular its most frequent subtype adenocarcinoma (56, 79, 97).

IV. MOUSE MODELS

A. Embryogenesis

In an attempt to define the physiological function of LKB1 in the mammalian setting, several laboratories have created mice carrying mutations targeted to the Lkb1 locus. Data show that functional Lkb1 is required for normal mouse embryogenesis. Lkb1 null mice do not survive beyond embryonic day (E) 10.5 and show several developmental abnormalities including impaired vascular development and shortened body axes (134). A role for increased vascular epidermal growth factor (VEGF) production in relation to the vascular abnormalities in these embryos has been discussed (134). Lkb1−/− mice survive to a stage of embryogenesis resembling approximately E7.5, passing through major developmental events such as implantation and gastrulation. The fact that Lkb1 null mice survive to a late stage of embryogenesis stands in clear contrast to the consequences of targeted biallelic Lkb1 recombination at later stages of development. Here, loss of Lkb1 in, for example, neural or pancreatic progenitors has clear cell-autonomous deleterious effects associated with a loss of cell polarity (12, 48). This puzzling context-dependent effect of the consequences of Lkb1 removal remains hitherto unexplained.

B. Polyp Development

Lkb1 heterozygous mice have proven instrumental in modeling the role of Lkb1 during PJS polyp development and tumor progression in the mammalian setting. Mouse models established in different laboratories have shown that biallelic Lkb1 inactivation is not required for polyp development (9, 57, 85, 92, 127). Lkb1+/− mice develop polyps that histopathologically mirror their human counterpart. Polyps retain the wild-type Lkb1 allele, showing that Lkb1 is haploinsufficient for suppression of polyposis. Polyps are first detected at around 5 mo and cause premature lethality from 8 mo onwards, presumably due to intestinal blockage. Polyps are particularly prominent in the stomach, in line with recent reports on polyposis in the human stomach (59, 123). Recent data show that neither a p53+− nor a p53−/− background allows for malignant transformation of murine Lkb1+/− polyps (116, 127). Moreover, while dimethylbenz[a]anthracene(DMBA)-treated Lkb1+/− mice (in a systemic carcinogen protocol) develop squamous cell carcinomas of the lung and skin, the number and histopathological features of the polyps remain unchanged (39). These observations suggest that murine PJS polyps carry little malignant potential.

The issue of polyp development in PJS patients has been surrounded by a long-standing debate concerning the potential for malignant degeneration of PJS polyps. Regardless of whether the polyps are ultimately deemed to lack malignant potential, it is clear that PJS polyp development and neoplastic transformation must both be accounted for by the same genetic mechanism, underscoring the need to elucidate polyp development in PJS.
The demonstration of loss of the wild-type \textit{LKB1} allele in human PJS polyps by CGH analysis initially inspired the proposal of the hamartoma-carcinoma sequence as the mechanism of tumor formation in PJS (17). This concept implied that PJS polyps, or hamartomas, are prone to malignant degeneration, in spite of their benign appearance, and that \textit{LKB1} acted as a classical tumor suppressor requiring biallelic inactivation for phenotypic expression. Recently, we proposed that PJS polyps are not pre-malignant and are a signpost to the malignant condition, not its direct obligate histological precursor (54). Polyps both in patients (30, 47) as well as in murine models (9, 57, 85, 92, 127) retain the wild-type allele. Moreover, polyps removed from patients are polyclonal (30). These observations do not accord with a clonal expansion scenario and argue in favor of a lack of malignant potential of PJS polyps.

Interestingly, a recent study involving \textit{Lkb1} recombination limited to the mesenchymal compartment suggests that polyp development results from defective epithelial-mesenchymal cross-talk, implying a “landscaper” scenario for PJS tumorigenesis (64). Irrespective of whether one or both “floxed” \textit{Lkb1} alleles were deleted upon expression of a \textit{Cre} transgene driven by the smooth muscle actin (\textit{Sm22}) promoter, polyposis ensued that was histopathologically indistinguishable from the polyps encountered in previous nonconditional \textit{Lkb1} \(+/-\) models (59). Due to reasons that are currently unclear, polyp burden was lower in these mice carrying conditional \textit{Lkb1} alleles compared with polyp burden in previous nonconditional models. The mechanism underlying polyp formation in this model has been suggested to involve a lack of secretion of growth and migrational stimuli such as transforming growth factor (TGF) and platelet-derived growth factor (PDGF) by \textit{Lkb1} \(+/-\) cells in the mesenchyma (59, 124).

In a second study employing a similar strategy, endothelium-specific \textit{Lkb1} removal using the \textit{Tie1-Cre} allele revealed that \textit{Lkb1} \(-/-\) yolk sac vessels in murine embryos appeared dilated and distorted. This defect in vascular remodeling was traced to a lack of recruitment of vascular smooth muscle cells due to defective TGF signaling by the \textit{Lkb1}-negative endothelium. A landscaper role for LKB1 in PJS suggests that PJS polyp development and malignant transformation occur due to inappropriate proliferative stimulation of the overlying epithelial compartment by the surrounding mesenchyma. Studies investigating a landscaper role for the LKB1 tumor suppressor should be predicated on the demonstration of the presence of both wild-type alleles in the overlying epithelium. The implications of this novel concept with regard to the debate surrounding the malignant potential of PJS polyps and the role of LKB1 in sporadic tumor development remain currently unclear.

C. Tumor Models

Models investigating the role of murine \textit{Lkb1} during malignant tumor progression have firmly established its tumor suppressive qualities. One study has reported high numbers of hepatocellular carcinoma (>70%) in aged \textit{Lkb1} \(+/-\) mice, but this has not been confirmed in other models, nor is hepatocellular carcinoma part of the PJS tumor spectrum (87). More recently, female \textit{Lkb1} \(+/-\) mice have been reported to spontaneously develop well-differentiated endometrial carcinomas with long latency, which are potentially analogous to adenoma malignum in female PJS patients (27). Conditional biallelic \textit{Lkb1} inactivation has been shown to result in neoplastic transformation in a number of tissues including lung (56), epidermis (39), pancreas (48), endometrial lining (27), and prostate (90). It is possible that the relative paucity of neoplastic transformation in \textit{Lkb1} \(+/-\) mice not bearing conditional alleles is due to the fact that these mice often develop fatal PJS polyps at an early age. Crosses of \textit{Lkb1} \(+/-\) and \textit{p53} \(+/-\) and \textit{p53} \(-/-\) mice have shown that these mice are highly tumor-prone, show an accelerated tumor-onset, and typically develop neoplasms part of the \textit{p53} tumor spectrum (sarcomas, lymphomas) (116, 127).

D. LKB1 Dosage Influences Cancer Susceptibility

By definition, LKB1 is haploinsufficient for the development of PJS, given that hemizygosity leads to characteristic phenotypes including gastrointestinal and nasal polyposis (61). With respect to its role as a tumor suppressor, recent studies now indicate that LKB1 dosage may critically influence cancer susceptibility. A most recent study investigating the role of \textit{Lkb1} in pulmonary carcinogenesis has shown that monoallelic \textit{Lkb1} inactivation cooperates with K-Ras mutation. Here, \textit{Lkb1} hemizygosity facilitated transformation and tumor metastasis, whereas biallelic inactivation of a conditional \textit{Lkb1} allele was associated with shorter latency and a different histological spectrum (56). Critically, \textit{Lkb1} inactivation altered the resulting spectrum of tumor histologies; mice carrying conditional \textit{Lkb1} alleles develop adenocarcinoma, squamous carcinoma, and large cell carcinoma, whereas tumors in other genetic backgrounds are strictly adenocarcinomas. In mice carrying a single conditional \textit{Lkb1} allele, the wild-type allele was apparently retained. This is similar to what has been observed on several occasions in murine models in other studies (27, 127) as well as in material derived from PJS patients (61, 101). Loss of the wild-type allele may thus afford additional selective advantages, but \textit{Lkb1} hemizygosity is clearly not neutral. Other well-known tumor suppressors such as PTEN have also demonstrated a context-dependent out-
come with regard to gene dosage (96). This implies that LKB1 may not adhere to the classical premise of tumor suppression, wherein both copies of a given tumor suppressor gene must be affected in order for malignant transformation to arise. Future research will be aimed at resolving this issue (102).

V. MOLECULAR CHARACTERISTICS OF LKB1

The LKB1 gene is ubiquitously expressed in both adult and fetal tissues (1, 60). The gene spans 23 kb and is composed of 10 exons, 9 of which are coding. It is transcribed in the telomere-to-centromere direction and encodes a highly conserved 50-kDa serine/threonine kinase. Alignment studies show that LKB1 has no close relative within the human genome, which similarly pertains to the mouse, fly, and worm genome. The catalytic domain shows the highest degree of conservation. Interestingly, inactivating mutations both from PJS patients and sporadic cases are not restricted to the kinase domain (1, 60).

Human LKB1 is a 436-amino acid protein that consists of an NH$_2$-terminal noncatalytic domain, a kinase domain (residues 49-309) that is most similar to the SNF1/AMP-activated protein kinase family, and a putative COOH-terminal regulatory domain. Endogenous and transfected LKB1 is present both in the nucleus and cytoplasm of cells. Two potential nuclear localization signals (NLS) are located at amino acids 38–43 and amino acids 81–84 (1, 108). A number of residues on LKB1 are either autophosphorylated (Thr-185, Thr-189, Thr-336, and Ser-404) or phosphorylated by upstream kinases (Ser-31, Ser-325, Thr-366, and Ser-431), and the residues surrounding these sites are highly conserved in Drosophila, Xenopus, and mammalian LKB1. Thus far, there are no reports showing that mutating these phosphorylation sites to Ala or Glu has an effect on kinase activity (18, 99, 100). However, changing Ser-431 into an Ala resulted in an LKB1 mutant, which is retained in the nucleus (109), suggesting that protein kinase C (PKC)-ζ, protein kinase A (PKA), or ribosomal protein S6 kinase (RSK) could regulate the localization of LKB1 by phosphorylating Ser-431 (99, 100, 109) and thereby stimulate the active transport of LKB1 out of the nucleus (33).

The COOH terminus of LKB1 contains a so-called CAAX-box, a consensus sequence for prenylation by addition of a farnesyl group allowing for plasma membrane insertion. LKB1 is indeed prenylated in cultured cells at Cys-433 (26, 100) and has, at least in invertebrate systems, been shown to be targeted to the plasma membrane (78, 126). It is interesting to note that naturally occurring PJS mutations include stop mutations that would prevent translation of the last 20 amino acids (1). Additionally, point mutation of the residue homologous to Cys-433 in Drosophila constitutes an allele with severely reduced rescue activity (78). These data indicate that the extreme COOH terminus of LKB1 is important for its function. Prenylation of endogenous LKB1 and the functional consequence of this modification have thus far not been investigated in a mammalian setting.

Recently, a novel shorter splice variant of LKB1 has been described. This shorter variant carries a unique 39-residue COOH-terminal sequence lacking some of the known phosphorylation and farnesylation sites described above, which may imply alternative posttranslational regulation of this variant. Although the exact expression pattern needs to be further characterized, depletion of this shorter splice variant in mice revealed a role in spermiogenesis (32, 120).

VI. LKB1-STRAD-MO25 COMPLEX

Although LKB1 had previously been linked to a plethora of cellular processes, little was known about its physiological regulation. Major insight into its regulation came with the observation that the Ste20 adaptor protein STRAD binds LKB1, which stabilizes and activates LKB1 (6). Further biochemical studies on the complex led to the identification of the scaffolding protein MO25 as a third component of the trimeric LKB1-STRAD-MO25 complex (18, 22).

A. STRAD Isoforms

The human genome encodes two isoforms of STRAD termed STRAD$\alpha$ and STRAD$\beta$ (1). Both isoforms have been classified as “pseudokinases” as they lack several key catalytic residues. Out of 518 protein kinases encoded by the human genome, 48 have been classified as pseudokinases (77). Sequence analyses of these pseudokinases indicate that they lack at least one of three motifs in the catalytic domain that are essential for catalysis. STRAD carries mutations in the third Asp-Phe-Gly (DFG) motif in subdomain VII of the kinase domain. The aspartic acid in this motif binds the Mg$^{2+}$ that coordinate the $\beta$- and $\gamma$-phosphates of ATP in the ATP-binding cleft of nonmutated, active kinase domains (20). Structural analysis has also shown that STRAD carries many inactivating mutations at its catalytic domain (82), and mutations aimed at restoring the catalytic activity of STRAD$\alpha$ by mutating residues back to those found in active kinases failed to reactivate STRAD$\alpha$ (20). Indeed, STRAD$\alpha$ does not autophosphorylate or phosphorylate any substrate tested (6). Curiously, although STRAD is an inactive pseudokinase, it still possesses several conserved motifs found in active protein kinases including the Gly-rich P-loop motif required for ATP binding. Indeed, STRAD does interact with ATP and ADP with high affinity ($K_d$ 30–100 $\mu$M) (20). The functional significance of this is
unclear, as mutations that prevent STRAD from binding ATP do not affect its ability to activate LKB1 or induce its cytoplasmic localization (1). It has been speculated that the STRAD proteins evolved from an active protein kinase that perhaps once controlled LKB1 (1). However, there is no evidence as yet to support this idea, as all vertebrate and sea urchin homologs of STRAD also lack catalytic residues (19). The NH₂- and COOH-terminal domains are not conserved between the STRAD isoforms, which may account for functional differences between the two proteins with regard to complex formation (33). In this respect, it is interesting to note that recently a novel condition designated PMSE due to a homozygous deletion in STRADα (or LYK5) has been described which presents with features of cortical thinning (91). Parents of patients are hemizygous for the allele but apparently did not present with any clinical abnormalities such as PJS polyps.

B. MO25 Isoforms

Similar to STRAD, there are also two isoforms of MO25, known as MO25α and MO25β (1, 18). Isoforms of MO25 interact with the COOH-terminal Trp-Glu-Phe residues (“WEF motif”) of STRAD and stabilize the interaction between LKB1 and STRAD (18). Structural studies have revealed that MO25α forms an extended α-helical repeat rodlike structure, distantly related to the armadillo repeat domain (82). At its COOH terminus, MO25α possesses a deep pocket that binds specifically to the WEF motif of STRADα, and mutation of this pocket inhibited the binding of MO25α to STRADα. Interestingly, a STRADα mutant lacking the COOH-terminal WEF motif can still form a complex with MO25α but only in the presence of LKB1 (18, 20). This indicates that interaction of LKB1 with STRADα creates an additional binding site for MO25 within the complex, which may explain why MO25α stabilizes the binding of LKB1 to STRADα (1). However, it will be necessary to crystallize the entire LKB1-STRAD-MO25 complex to understand in full the molecular mechanism by which these proteins interact.

C. Complex Formation and LKB1 Activation

Unlike most kinases, LKB1 is not activated by phosphorylation of its activation loop by an upstream kinase, but is instead activated upon binding to STRAD (6). Complexes of LKB1-STRAD-MO25 can be isolated from mammalian cells in which the three components are present in a similar stoichiometry (18). LKB1 relocalizes from the nucleus to the cytosolic compartment when coexpressed with MO25 and STRAD (6, 7, 22), and the amount of STRADα associated with LKB1 in cells is significantly enhanced by the expression of MO25α. In addition, siRNA-mediated knockdown of MO25α reduces the amount of endogenous STRADα associated with LKB1 (18). Note, however, that most of these cell culture experiments involve overexpression assays, and the physiological regulation of the localization and activity of LKB1 in vivo therefore remains unclear. The pseudokinase domain of the STRAD proteins binds directly to the kinase domain of LKB1, enhancing its catalytic activity over 100-fold. The molecular mechanism by which STRAD activates LKB1 has not yet been elucidated, but it is possible that their interaction leads to a conformational change that stabilizes LKB1 in an active conformation (1). Interestingly, several single amino acid substitution mutants of LKB1 isolated from patients have lost the ability to interact with STRAD (20), pointing to the importance of the STRAD-LKB1 interaction.

VII. THE METABOLIC AMPK MODULE IS A DOWNSTREAM TARGET OF LKB1

A. Physiological Regulation

Purification of the LKB1-STRAD-MO25 trimeric complex allowed for the characterization of the most prominent downstream LKB1 substrate identified so far in mammalian systems, the AMPK system (for review, see Ref. 41). AMPK has emerged as a master regulator of cellular energy metabolism with dozens of downstream targets...
(Fig. 3). Mammalian AMPK is a heterotrimeric complex consisting of catalytic α-subunits (α1 or α2), and regulatory β- (β1 or β2), and γ-subunits (either γ1 or γ2 or γ3). AMPK is activated by any cellular stressor that depletes cellular ATP. Activation of AMPK results in downregulation of ATP-consuming pathways, while switching on ATP-generating pathways (41). Activation of AMPK by AMP occurs in two ways: AMP activates AMPK directly via an allosteric mechanism by binding at the regulatory γ-subunit, as well as indirectly by inhibiting dephosphorylation of a regulatory “T-loop” threonine residue within the kinase domain at Thr-172 by protein phosphatases. The combination of the two results in >1,000-fold increase in kinase activity (112). The identity of the protein phosphatase(s) acting on AMPK in vivo is unknown; however, the effect of AMP on dephosphorylation is substrate mediated so AMP would be predicted to inhibit all phosphatases acting on AMPK. Moreover, a single point mutation in a domain of the regulatory γ-subunit abolishes both the inhibitory effect on dephosphorylation and the direct allosteric activation (98). This point mutation occurs in an autosomal dominant form in the Wolff-Parkinson-White syndrome, a heart disorder characterized by abnormal storage of glycogen in cardiac myocytes and ventricular preexcitation leading to disturbances in cardiac conduction and rhythm abnormalities (41). AMPK can be pharmacologically activated by AICAR (an AMP mimic after intracellular conversion to AICAR-monophosphate), phenformin, and metformin. The latter is a widely prescribed drug in the treatment of non-insulin-dependent diabetes mellitus, which is thought to exert its blood glucose-lowering effects through its ability to activate AMPK in skeletal muscle and liver (139).

AMPK activation in vivo has both short-term and long-term effects on energy metabolism. Acute effects include increasing glucose transport and activating fatty acid oxidation through phosphorylation of metabolic regulators. Repeated or prolonged activation of AMPK is associated with increased expression of enzymes involved in glucose and lipid oxidation and the mitochondrial electron transport chain (121). These effects might be established independently through either AMPKα1 or AMPKα2 isoforms (see below). With regard to metabolic adaptation through the induction of downstream metabolic targets, it was recently shown that AMPK activation through prolonged AICAR administration induced a metabolic gene expression profile in untrained mice (88). Remarkably, prolonged AICAR administration translated into an increased running endurance in sedentary mice. The role for LKB1 activation in these metabolic adaptations remains to be investigated, although LKB1 has been shown to be responsible for some of the downstream effects of AICAR, for example, on fatty acid oxidation (117). It will therefore be of particular interest to determine the effect of prolonged AICAR administration on AMPK activation, metabolic adaptation, and training endurance in conditional Lkb1 mouse models (see below).

B. LKB1 Is an AMPK Kinase

LKB1 was originally described as the upstream AMPK kinase in response to studies investigating the kinases responsible for activation of the yeast AMPK ortholog snf1 (1). There are no clear orthologs of these upstream kinases in the human genome, but one of the protein kinases with a kinase domain closest to these upstream kinases in yeast is human LKB1. Following this observation, LKB1 was shown to efficiently phosphorylate AMPK at its regulatory T-loop residue Thr-172 in biochemical assays (42, 104, 132). A requirement for MO25 and STRAD in the process was shown by demonstrating that the ability of LKB1 to activate AMPK was greatly enhanced when present in a trimeric complex. Finally, it was shown that AMPK could not be activated by known activators of AMPK such as phenformin or AICAR in cell lines that lack LKB1 (42, 104, 132). AMPK activation was restored by stably expressing wild-type, but not catalytically inactive, LKB1 (42). These cell lines do, however, display a basal level of AMPK activation (42, 104), hinting at the possibility that LKB1 is not exclusively responsible for AMPK activation. Other kinases such as the calmodulin-dependent protein kinase kinases (CaMKKs) and TAK-1 have since been shown to function as upstream kinases for the AMPK complex (41). This might explain why loss of AMPK activation is not a universal feature following conditional Lkb1 removal in mice (12). LKB1 can phosphorylate both AMPKα1 and AMPKα2 with similar efficiency in vitro (42). Studies that suggest that the LKB1 complex is constitutively active in vivo, since treatments that activate AMPK apparently do not change LKB1 catalytic activity (76, 94). Encompassing data described above showing that AMP activates AMPK by allosteric activation as well as by protecting the regulatory T-loop residue from Thr-172 dephosphorylation suggest a model for AMPK activation wherein the phosphorylation state of Thr-172 depends on the relative rates of phosphorylation, catalyzed by upstream kinases such as LKB1 and CaMKKs, and dephosphorylation, catalyzed by protein phosphatases that remain to be further characterized (98).

C. LKB1 Exhibits Isoform Specificity Towards AMPK

Recent studies have painted a particularly interesting picture with respect to the differential regulation of AMPK isoforms by LKB1. In a conditional Lkb1 mouse model, it was shown that in muscle lacking Lkb1, neither contraction nor AICAR activates the AMPKα2 isoform.
(95). In this model, complete Lkb1 loss occurred only in skeletal muscle through the expression of a Cre recombinase driven by the muscle creatine kinase promoter, whereas other tissues such as testis, kidney, and lung express ~10% of the normal level of Lkb1 due to expression of a hypomorphic allele. In these mice, Thr-172 phosphorylation of the AMPKα1 isoform in response to prolonged overload increased similarly in normal and Lkb1-depleted skeletal muscle; Thr-172 phosphorylation of AMPKα2 was not increased in control animals and completely absent in the Lkb1-depleted mice (81). These results suggest that in Lkb1-deficient muscle, the AMPKα1 isoform is activated at the Thr-172 T-loop residue normally in response to chronic overload. Thr-172 phosphorylation of the AMPKα2 isoform appears not to be involved in chronic overload responses such as muscle hypertrophy (81). Therefore, it is becoming increasingly clear that the AMPKα1 and AMPKα2 isoforms have distinct regulatory properties and functions in vivo and show discordant regulation in response to metabolic stimuli (Table 2). It has been proposed that the Lkb1-regulated AMPKα2 isoform is involved in metabolic adaptations. Thus diminished AMPKα2 isoform activation might be expected to reveal itself predominantly in metabolic phenotypes such as altered glucose regulation. This is in line with the fact that knockout of the AMPKα1 isoform in mice did not result in a detectable metabolic phenotype, whereas AMPKα2 isoform knockout mice exhibit insulin resistance (58, 125). Note that these mice do not exhibit any of the abnormalities associated with PJS, in particular PJS polyposis, although tissue-specific redundancy cannot be ruled out. Conversely, PJS patients have not been reported to demonstrate any metabolic or endocrine abnormalities.

Lkb1 depletion targeted to the liver has been shown to result in reduced total hepatic AMPK activity and hyperglycemia (105). This was suggested to result from uninhibited gluconeogenesis due to unabated activation of the transcriptional coactivator Torc2. Moreover, hyperglycemia in mice lacking hepatic Lkb1 expression did not respond to the blood glucose-lowering effects of metformin treatment, indicating that Lkb1-mediated activation of AMPK in the liver is required for the ability of metformin to lower blood glucose levels (105). Strikingly, a recent study reported improved glucose tolerance and insulin sensitivity after Lkb1 depletion restricted specifically to skeletal muscle (68). Thus loss of Lkb1 in either liver or skeletal muscle leads to opposite effects on systemic glucose regulation. This might be explained by the fact that hepatic deletion of Lkb1 leads to increases in PGC1α (105), whereas skeletal muscle-specific Lkb1 depletion decreases PGC1α (68). PGC1α is thought to mediate transcription of key gluconeogenic enzymes (81). Curiously, the Lkb1 mice that have an ~90% Lkb1 reduction in many tissues and ablation of Lkb1 activity in skeletal muscle were reported to exhibit normal blood glucose concentrations (95). It seems plausible that in this case the beneficial effects of a reduction in skeletal muscle Lkb1 expression on glucose homeostasis are masked by the detrimental effects of a reduction in hepatic Lkb1 expression. Collectively, these studies point to a role for Lkb1 in metabolic regulation, predominantly through the AMPKα2 isoform.

D. mTOR Module

Particularly well-established downstream effects following AMPK activation are mediated through the TSC1/TSC2-mTOR pathway, which elicit an inhibitory effect on protein translation (41). AMPK phosphorylates and activates TSC1/TSC2, which suppresses the activity of the small GTPase Rheb. Active GTP-bound Rheb normally activates mTOR. However, TSC1/TSC2 activation switches Rheb off through GDP conversion, thereby inactivating mTOR. This leads to dephosphorylation of the physiological mTOR effectors S6K and 4E-BP1, which are involved in translation initiation. Recently, Raptor has been identified as another downstream effector of AMPK that may elicit a cellular energy stress program by inhibiting the mTOR module (40).

Previously, studies had suggested a role for the mTOR module in Lkb1 tumor suppression (1). This was shown, at least partly, by the fact that mutations in TSC1

### Table 2. Overview AMPKα1 and AMPKα2 isoforms

<table>
<thead>
<tr>
<th></th>
<th>AMPKα1</th>
<th>AMPKα2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>62.8 kDa/550 amino acids</td>
<td>62.3 kDa/552 amino acids</td>
</tr>
<tr>
<td>Phosphorylation sites</td>
<td>Thr-171 (LKB1, CAMKKs), Thr-258, Ser-458</td>
<td>Thr-171 (LKB1, CAMKKs), Thr-258, Ser-491</td>
</tr>
<tr>
<td>Tissue distribution</td>
<td>Activity ubiquitous</td>
<td>Activity highest in skeletal muscle, liver, and heart</td>
</tr>
<tr>
<td>Subcellular localization</td>
<td>Cytoplasmic</td>
<td>Nuclear, cytoplasmic</td>
</tr>
<tr>
<td>Upstream activators</td>
<td>LKB1 complex, CAMKKs</td>
<td>LKB1 complex, CAMKKs</td>
</tr>
<tr>
<td>Activators</td>
<td>AICAR, metformin, A-769062</td>
<td>AICAR, metformin, A-769062</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>Compound C*</td>
<td>Compound C*</td>
</tr>
<tr>
<td>Nuclear localization signal</td>
<td>No</td>
<td>Yes (residues 218–231) (114)</td>
</tr>
<tr>
<td>Mouse model</td>
<td>Knock-out displays no phenotype (58)</td>
<td>Insulin resistant, glucose intolerant (125)</td>
</tr>
</tbody>
</table>

Reference numbers are given in parentheses. * Note that compound C targets a broader range of kinases and is not a specific AMPK inhibitor (8).
LKB1-AMPK FAMILY SIGNALING

and TSC2 are found in tuberous sclerosis, a disease that features hamartomatous tumor formation in various organs. PJS polyps did show increased mTOR activation as is evident from increased phosphorylation of its targets SGK and 4E-BP1 (103). However, increased mTOR activation occurs in many instances of increased proliferation of which the mature PJS polyp is but one example. Moreover, there is little clinical overlap between PJS and tuberous sclerosis, as stated above. A recent study investigating the effect of the mTOR inhibitor rapamycin in the Lkb1 +/− mouse model found that rapamycin treatment affects PJS polyposis by inhibiting the growth of established polyps, whilst smaller polyps were unaffected (128). This might be explained by the fact that rapamycin mainly inhibits proliferation, whereas it may not affect the underlying mechanism of polyp formation. Moreover, activation of TSC2 and TORC1 is not affected by Lkb1 depletion in skeletal muscle (81). Evidence for a role of mTOR in LKB1 signaling has remained scarce, and with the demonstration of a role for AMPK in polarity regulation (see below), in addition to its well-described role in energy metabolism, the latter pathway has since come under increased scrutiny.

E. Clinical Applications

Following the demonstration that the tumor suppressor LKB1 is upstream of AMPK, it has been suggested that the inhibition of cellular proliferation through AMPK activation may be used to prevent or even treat neoplastic transformation (1). Retrospective epidemiological studies have suggested that diabetics on metformin have a reduced likelihood of developing cancer (21, 35). Likewise, AICAR treatment of cell lines in culture was shown to inhibit proliferation (115). Recently, in an in vivo model, activation of the AMPK pathway by metformin, phenformin, or A-769662 treatment was shown to significantly delay tumor onset in Pten +/- mice also carrying a hypomorphic mutation in Lkb1 (50). LKB1 was shown to be required in vitro for activators of AMPK to inhibit cellular proliferation in PTEN-deficient cell lines (50). Although the concept of suppressing tumor development by triggering a physiological signaling pathway that functions as a cellular energy-sensing checkpoint appears attractive, it needs to be remembered that AMPK as stated previously displays a broad range of cellular targets (41). Indeed, switching on a cellular energy checkpoint may also trigger undesirable responses such as enhanced angiogenesis. Furthermore, low-energy conditions may make tumor cell populations less susceptible to standard treatments that depend on cellular turnover such as radiation therapy or chemotherapy. Thus future studies should investigate whether any of these pharmacological modalities such as metformin (already in clinical use for the treatment of diabetes mellitus) can translate into a significant benefit in the oncological setting.

VIII. LKB1 AND CELLULAR POLARIZATION

Before the discovery of the energy-sensing AMPK module as a downstream target, LKB1 had been most prominently linked to the regulation of cellular polarization in model organisms. The allele encoding the Caenorhabditis elegans counterpart of the LKB1 tumor suppressor Par-4 was initially retrieved in screens designed to pick mutants defective in asymmetric cell division of the fertilized worm zygote. C. elegans Par-4 mutants show symmetric cleavage patterns and absence of gut granules showing that Par-4 is crucial for establishing cell polarity during asymmetric cleavage patterns of blastomeres and specification of the intestinal lineage (126). Likewise, the Drosophila LKB1 counterpart dLkb1 was retrieved by screening for mutants defective in anterior-posterior oocyte axis formation (78). These studies remain some of the strongest evidence linking LKB1 to cellular polarization and facets thereof. This role of LKB1 and recent studies substantiating this role will be discussed next. We will examine different canonical polarity model systems, highlighting recent studies that have shown a surprising convergence of LKB1-AMPK signaling on polarity establishment.

A. A Family of Par Proteins Regulates Cellular Polarity

The acquisition and maintenance of cellular polarity is a fundamental process both during development and during adult tissue homeostasis. It has been found that a core set of Par (for “partitioning defective”) proteins, along with a limited number of other proteins such as aPKC, are involved in a broad range of phenomena requiring proper cellular polarization (37). On the basis of work performed initially in C. elegans employing genomic screens looking for mutants demonstrating symmetric first cleavage patterns (62), a family of six Par genes has been cloned and characterized. In this family Par-1 and Par-4 encode serine threonine kinases; Par-5 is a member of the family of 14-3-3 proteins; Par-3 and Par-6 contain PDZ domains, suggesting that they may act as part of a signaling scaffold; and, finally, Par-2 displays a RING finger domain which suggests it may act in a ubiquitination pathway. The latter protein is not conserved beyond C. elegans, however. This core module of Par proteins appears to be recursively recruited in several contexts of cellular polarization, such as neurite extension, cellular migration, and asymmetric cell division. A complex epistatic relationship for members of this family has been

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revealed by a host of functional studies (37). LKB1/Par-4 may reside at the top of a cascade regulating the asymmetric segregation of Par family proteins, since LKB1/Par-4 mutations are epistatic to all other Par family mutants (60). Combining data from several model systems suggests a model wherein antagonistic activities of the different Par modules regulate the identity of different cellular membrane domains (Fig. 4) (37). In this scheme, Par-1/MARK may phosphorylate Par-3 on two distinct serine residues generating binding sites for Par-5/14-3-3, which inactivates Par-3 and prevents it from invading the basolateral domain by inhibiting Par-3/Par-6/aPKC complex formation (13). Conversely, aPKC may phosphorylate Par-1/MARK, which allows for 14-3-3 binding and inhibits its function at the apical domain. Since LKB1 sits at the top of this cascade, its function may be to “tilt the balance” between these opposing kinase modules in different membrane domains or in different contexts. Indeed, it has been shown that LKB1 can phosphorylate Par-1, resulting in the activation of this kinase (see below) (76, 110). 14-3-3 is required for reciprocal exclusion of both modules and is therefore not asymmetrically localized (37). Whether a similar mode of operation applies for LKB1, which also appears not to be asymmetrically localized, remains to be investigated.

Note that in some instances significant differences between mechanisms of cellular polarization have been observed between invertebrate and vertebrate model systems. It is clear, however, that similar to the mechanisms deployed during asymmetric cell division in C. elegans and Drosophila, progenitor cells within mammalian stem cell niches, such as the interfollicular epidermis (70) and the subventricular zone in the developing brain (for review, see Ref. 67), recruit Par family members to divide asymmetrically. Studies that have begun to unravel the signaling network downstream of LKB1 indicate that it may signal to a host of intracellular targets depending on context.

B. LKB1 in Epithelial Polarity

The formation of cellular sheets displaying apical-basal polarity is a defining attribute of several epithelial tissues. Epithelia often perform a dual barrier function with the environment, permitting the regulated uptake or excretion of substances at its apical surface, whilst maintaining a closed, impenetrable surface through the formation of tight intercellular junctions. Notable examples of this form of polarization include the intestinal epithelial lining displaying apical brush borders and the ciliated epithelium of the lung. Similar to the antagonistic modules described above, cellular polarization in mammalian cell culture systems has been shown to proceed through mechanisms whereby membrane domains are established through mutual exclusion driven by phosphorylation, 14-3-3 association, and competitive binding (37, 52, 113). Evidence on the domineering role played by LKB1 in epithelial polarization was provided in a study showing that single intestinal epithelial cells polarize in a cell-autonomous fashion in response to the activation of LKB1 (7). Cellular polarization is accomplished through the induction of STRAD expression in these cells, which allows for stabilization and activation of LKB1. Cells polarize within hours after STRAD induction in the absence of neighboring contacts. Polarized cells display several defining features of polarized intestinal epithelial cells, including reorganization of the actin cytoskeleton to form an apical brush border, relocalization of junctional ZO-1 proteins to facilitate directed endosomal transportation (7). These observations suggest that LKB1 acts upstream of the other Par members in mammalian epithelial cells, similar to genetic data retrieved from Drosophila and C. elegans. For instance, the observation that putative junctional com-

FIG. 4. Par proteins establish cortical identity. From studies in Drosophila, C. elegans, and mammalian cell culture systems, it has become clear that various aspects of cell polarity are regulated by an evolutionarily conserved module of Par (“partitioning defective”) proteins, which is recursively iterated in various contexts of cellular polarization. Antagonistic phosphorylation events regulate the mutual exclusion through 14-3-3 association and segregation of Par proteins to different cortical domains. LKB1/Par-4 is a pivotal upstream regulator of cellular polarization.
plexes form in the absence of neighboring cells indicates that LKB1 can regulate Par-3 and Par-6 by establishing the structures that recruit this complex. Obviously, this does not exclude other pathways that emanate from LKB1 to regulate the Par-3 complex, or vice versa. The exact role of 14-3-3/Par-5 in this context remains unclear; recent work indicates that 14-3-3ζ can interact with phosphorylated MARK family members, thereby blocking plasma membrane localization of the latter (38).

Studies examining epithelial polarization in the MDCK cell culture model system have provided evidence for a remarkable role of AMPK activation in polarity establishment, in addition to its role in energy metabolism. In mammalian MDCK cell culture models, activation of AMPK appears to be required for repolarization of MDCK cells in response to changes in extracellular calcium (“calcium switch” model) (136, 138). Experiments using kinase-dead LKB1 showed that activation of AMPK during cellular repolarization was dependent on LKB1. This work did not investigate whether expression of kinase-dead LKB1 translated into polarization defects (136).

Note that, in contrast to studies demonstrating a differential regulation of the AMPKα1 and AMPKα2 isoforms in skeletal muscle by Lkb1, these studies have not examined whether in epithelial cells the various AMPKα isoforms are differently involved. Important support for these observations in mammalian cell culture models has now come from two studies examining the phenotype of the Drosophila AMPK null mutant. Mutants were embryonic lethal and showed extensive defects in cellular polarization as shown by the mislocalization of specific membrane markers (72, 84). Thus AMPK signaling in polarity establishment appears to be evolutionary conserved. Active mutants of myosin regulatory light chain (MRLC) rescued many of the defects associated with AMPK loss, suggesting that MRLC is principally responsible for executing signaling downstream of AMPK in this context (72). This is a remarkable finding as AMPK displays an array of downstream targets. This would suggest a conserved role for actomyosin contractility in cellular polarization (28). With regard to the regulation of MRLC in mammalian systems, activation of AMPK has been shown to correlate with MRLC inhibition rather than activation in vascular smooth muscle cells (49), and deletion of Lkb1 in pancreas did not affect MRLC phosphorylation status (48). Further research will therefore be required to resolve these issues.

Following the identification of LKB1 as the upstream kinase of AMPK, it was quickly realized that LKB1 might activate other kinases akin to AMPK. Indeed, LKB1 was shown to function upstream of 12 additional kinases that fall on the same proteomic branch as AMPK by phylogenetic analyses (1, 76). These kinases are all activated by LKB1 through phosphorylation of their “T-loop” threonine residue, which is required for full activity (76). This multitude of downstream LKB1 substrates now implicates LKB1 signaling in an array of cellular processes ranging from metabolic control to cellular polarization. As observed for AMPK, STRAD and MO25 are essential for LKB1 to phosphorylate AMPK-related enzymes. LKB1 does not activate all kinases in this branch, and specificity appears to be partly mediated by a preference for a leucine at position −2 relative to the T-loop. Among the kinases in this family of AMP kinases are the four mammalian homologs of the Par-1 kinase, MARK1–4 (for microtubule affinity-regulating kinase 1–4) and the BRSK1/SAD-A, BRSK2/SAD-B kinases. The MARK kinases will be discussed next, and the BRSK1/SAD-A, BRSK2/SAD-B kinases will be discussed more extensively thereafter. For a more comprehensive review on the other downstream AMPK family substrates of LKB1, the interested reader is referred to Reference 1.

Extensive evidence exists to support a conserved function of the MARK family members in regulating aspects of mammalian epithelial polarization. The four closely related MARK kinases are the mammalian counterparts of the Drosophila and C. elegans Par-1 kinase, which function cooperatively with LKB1/Par-4 in the establishment of cellular polarity in these model organisms (14, 78). Previous studies indicated that the four members of this AMPK-related kinase subfamily play similar roles in regulating cell polarity at a mammalian level (15, 25, 34, 113). The demonstration that MARK isoforms are phosphorylated and activated by LKB1 (76, 110) suggested that LKB1/Par-4 regulates cell polarity by activating MARK/Par-1 isoforms. These phosphorylation events may lead to the establishment of distinct cellular membrane domains through mutual exclusion with the Par-3/6 module according to the scheme outlined above. Proteomic analyses suggest that this network is conserved at the mammalian level (22).

In mammalian cells, MARK isoforms are a major determinant of the organization of the microtubule skeleton through the phosphorylation of microtubule-associated proteins (34). This may cause detachment of the latter from microtubules and induce microtubule destabilization (15). Thus MARK activation may locally regulate microtubule density and mediate critical aspects of cellular polarization, such as centrosome repositioning. In an elegant study by Taketo and co-workers (69), LKB1 depletion was found to affect microtubule dynamic instability by suppressing microtubule polymerization through activation of the AMPK family member MARK2 (69). This work accords with earlier data from the Drosophila system, wherein germline dLkb1 clones show loss of the polarized microtubule network in the Drosophila oocyte (78). Two somatic point mutations of MARK3 have recently been reported in sporadic colorectal cancer (89), suggesting that MARK3 may be causally involved in tumorigenesis. The genes encoding these MARK isoforms...
would represent potential secondary PJS loci; however, mutation analysis did not reveal germline mutations in PJS patients not previously linked to LKB1 mutations (29). In addition to LKB1, another kinase named TAO1 was shown to activate MARK2 by T-loop phosphorylation (119). This suggests that LKB1 may not exclusively mediate activation of downstream substrates in the MARK family, as has similarly been shown for CAMKK and TAK1 with regard to AMPK. The contribution of the MARK family members to the establishment of cellular polarization remains under investigation. MARK knockout mice have been established, but these did not reveal obvious defects in cellular polarization, although redundancy among the four family members may have masked functional defects in this respect. Strikingly, MARK2 (also called Par-1b) knockout mice have recently been found to be hypoinsulinemic and thus display a metabolic phenotype (51). These mice had increased glucose tolerance but were normoglycemic under fasting conditions. This indicates that MARK family members may function in the regulation of metabolic homeostasis in addition to cellular polarization, echoing results obtained for the AMPK module.

C. LKB1 in Neuronal Polarity

Recent studies have examined the role of LKB1 in neuronal polarization and found additional downstream substrates. In the developing neocortex, neural progenitors migrate away from their birthplace within the ventricular zone to differentiate and establish synaptic connections in the cortical plate by assuming proper axonal/dendritic polarity. Neuronal migration and differentiation are dependent on a coordination of events, involving neurite outgrowth and centrosome relocation. Neurite extension is a classic model for research on mechanisms of cellular polarization, and a pivotal role for Par family members has been demonstrated in this system as well (37). Loss of a conditional Lkb1 allele in telencephalic neuronal progenitors impairs corticofugal axon extension, resulting in cortical thinning and agenesis of the corpus callosum in mice (12). This phenotype was traced specifically to a defect in activation of the Brsk/Sad kinases, which appear to be responsible for cytoskeletal reorganization through tau phosphorylation (12). Previously, Sad-A/Sad-B combined knockout mice had shown a similar phenotype of reduced axon outgrowth and mislocalization of axon and dendritic markers (65). These SAD kinases are expressed mainly in the brain and are members of the AMPK family of downstream LKB1 effectors, which are phosphorylated on the T-loop residue in the activation domain like the Par-1/MARK subfamily of kinases (see above). No changes in AMPK phosphorylation in Lkb1-deficient cortex were noted in this study (4, 111). Additionally, extracellular cues such as brain-derived neurotrophic factor (BDNF) were shown to induce axon formation in vitro by locally activating LKB1 phosphorylation on serine-431 (130). A third study took a similar approach using in vivo electroporation of RNAi constructs targeting Lkb1 transcripts into neonatal mouse cortex to investigate the effects of Lkb1 depletion. Here, it was similarly found that neuronal migration and axonal polarity were impaired, which were linked to a malpositioning of the centrosome in migrating and differentiating neurons. LKB1 has similarly been found to affect polarized migration in response to wound healing in cell culture models of migrating epithelial cells, which may be regulated through the small GTPase Cdc42 (137). Polarized migration involves major cytoskeletal reorganization events, involving Golgi and centrosome repositioning and lamellipodia formation. Localized LKB1 activation and subsequent microtubule reorganization, through for example tau phosphorylation (69), may depend in vivo on neurite outgrowth-promoting cues such as BDNF (12). A role for AMPK in polarized migration is conceivable regarding its instructive role in the establishment in epithelial polarity across systems, although evidence for such a role remains scarce at this point. Collectively, current data make it clear that Lkb1 deficiency in cortical neurons leads to a cell-autonomous defect in axon formation and appear to reaffirm the critical role of LKB1 in polarity regulation in vivo. A key challenge of future research will be to identify the substrates BRSK/SAD and MARK kinases phosphorylate to regulate cell polarity in vivo. Clearly, the role of LKB1 during cellular polarization is not limited to epithelial systems.

D. Role of LKB1 in Asymmetric Cell Division

The work discussed so far examined a role for LKB1-AMPK signaling in cell polarization during interphase. However, a large body of work has documented a role for LKB1 during mitosis as well. Cell division involves many aspects similar to interphase cellular polarization, and similar signaling pathways will therefore be reiterated. In fact, the original Par-4 allele, which allowed for the cloning of the C. elegans LKB1 homolog, was retrieved in screens examining asymmetric cell division long before downstream LKB1 effectors such as the AMPK family members were functionally characterized (62). The Par genes that were retrieved in C. elegans are required for two related aspects of cell polarization during asymmetric cell division: the asymmetric placement of the mitotic spindle, which results in unequal division, and the asymmetric positioning of factors determining cell cycle timing and cell fate distinction between daughter cells, the so-called cytoplasmic determinants (Fig. 5) (14). Interestingly, Par-4 mutants do show asymmetric first cleavage
patterns, albeit with larger variation (86, 126). Divisions in the two-cell Par-4 embryo occur with near-perfect synchronicity, which is in stark contrast to the wild-type embryo, wherein divisions are strictly asynchronous. Thus Par-4 does act during the first cell cycle, as mutants do not segregate cytoplasmic determinants at this time even though the actual cleavage pattern is asymmetric. Par-4 therefore demonstrates an uncoupling of spindle placement and determinant segregation during asymmetric cell division in the C. elegans zygote. In Drosophila, oocyte polarization occurs before, not during, fertilization as in C. elegans, yet with the exception of Par-2, which is not conserved in Drosophila, the same set of proteins appears to be involved (14). The Drosophila LKB1 ortholog dLkb1 has been found to regulate the establishment of oocyte polarization, and mutation of dLkb1 is embryonic lethal (78). The Drosophila Par-1/MARK homolog has also been found to cooperate with dLkb1 in this process (78, 118). Moreover, similar to Par-4 in C. elegans, a recent forward genetic screen in the Drosophila model has uncovered a role for dLkb1 during neuroblast asymmetric cell division (16). Neuroblast cell division represents one of the best-characterized model systems for asymmetric stem cell division (for a review, see Ref. 67), and key players recovered in this system (such as Lgl and Pins) have been found to play a conserved role during asymmetric cell division in higher organisms as well (66, 70). Asymmetric neuroblast division is characterized by the asymmetric inheritance of segregating determinants and the coupling of mitotic spindle orientation to determinant localization. Mutations in regulators of either process have been shown to result in a hyperproliferation phenotype due to occasional neuroblast divisions in mutant brains undergoing ectopic self-renewal through symmetric cell division (71). Mutation of the Drosophila LKB1 homolog resulted in disruption of many aspects of asymmetric larval neuroblast division, such as asymmetric microtubule length and centrosome size, and disruption of asymmetric distribution of lineage determinants. Neuroblasts mutant for dLkb1 were shown to undergo occasional ectopic symmetric divisions, and mutant brains demonstrate a phenotype of hyperproliferation, accordingly. The Drosophila STRAD and MO25 homologs were also shown to be involved in neuroblast division in a second study (133).

At this point, direct evidence for a role of LKB1 in mammalian asymmetric cell division has not been put forth. However, based on loss of function studies in invertebrate model systems, LKB1 may have a role in directing asymmetric cell division in higher organisms as well. Lineage determination in several adult mammalian stem cell compartments also proceeds via asymmetric stem cell division occurring in stem or progenitor cells (67). We have recently shown that normal unaffected intestinal epithelium in PJS patients shows an expanded progenitor compartment (30). Previous studies have indicated that the mammalian intestinal epithelium is maintained through asymmetric progenitor cell divisions in the intestinal stem cell compartment (10). In addition, progenitor cells undergo symmetric divisions during normal homeostasis, which may dictate the expansion or loss of intestinal progenitor cell lineages within the stem cell compartment (Fig. 6) (73). An expanded progenitor compartment as observed in normal unaffected Peutz-Jeghers epithelium may be due to occasional ectopic symmetric progenitor cell divisions, in accordance with the consequences of LKB1 mutation in invertebrate model organisms. Stochastic bursts of epithelial proliferative activity may form mucosal outpocketings upon which mechanical forces can act. Potentially these lesions wax and wane over time (smaller lesions may be extruded or auto-amputated) and fail to come to clinical attention (123). A “tug-of-war” between small mucosal elevations and mechanical forces may precipitate the mature irregular, cauliflower-like PJS polyp (Fig. 1), which is typically located at sites of greatest mechanical traction, such as the pylorus.

**FIG. 5.** Phenotypic summary of Par mutant embryos in C. elegans. The fertilized C. elegans zygote divides asymmetrically to generate two daughter cells of dissimilar developmental potential. Par mutations affect either the segregation of cytoplasmic determinants (“P granules”), the eccentric placement of the spindle, or both of these processes.
to accelerate the time to neoplastic transformation in
in PJS patients, an expanded progenitor pool is predicted
of transformation. With regard to the cancer-prone state
yps per se are ultimately deemed to lack an inherent risk
same genetic mechanism, regardless of whether PJS pol-
plastic transformation must both be accounted for by the
previously, it is clear that polyp development and neo-
transformative features. In the colon, this multistep progression
model follows a sequence of histopathological steps from
ormal epithelium to small adenoma, larger adenoma
with high-grade dysplasia, and eventually invasive carci-
. It remains unresolved whether PJS polyps carry an
inherent risk for neoplastic transformation (54). As stated
previously, it is clear that polyp development and neo-
plastic transformation must both be accounted for by the
same genetic mechanism, regardless of whether PJS pol-
ys per se are ultimately deemed to lack an inherent risk
of transformation. With regard to the cancer-prone state
in PJS patients, an expanded progenitor pool is predicted
to accelerate the time to neoplastic transformation in
normal unaffected epithelia (Fig. 6). In the colon, time to
gatekeeper mutation would therefore be decreased in PJS
patients at normal background mutation rates. In this
scenario, colorectal tumor progression in PJS patients
would be expected to adhere to a conventional adenoma-
carcinoma progression model; features of the mature PJS
polyp such as smooth muscle proliferation may be ac-
quired coincidentally later during adenoma development
(see sect. 4B on clinical characteristics). Although evi-
dence for this notion remains scarce at the moment, it has
been suggested that PJS patients indeed develop adenoma-
matous polyps at an increased rate compared with the
general population (80). In contrast, transgenic Lkb1
mouse models have not been shown to develop adenoma-
tous polyps so far. This may relate to the fact that these
mice succumb to intestinal blockage at a time before
adenomatous changes in the murine colon has had time
to evolve. Thus, with regard to tumor progression in the

![Fig. 6. Lineage competition in the intestinal stem cell crypt.](image)

A: model depicting asymmetric progenitor cell division. Stem cell lin-
eages may either be driven towards extinction or expansion by symmet-
ric progenitor cell divisions resulting in two daughter cells committed to
differentiate or two daughter cells retaining progenitor cell characteris-
tics, respectively. Asymmetric progenitor cell divisions leave the pro-
genitor cell population unchanged. B: clonal evolution in stem cell compartments. Depicted is a hypothetical progenitor compartment (a
“niche”), which contains two progenitor lineages at baseline. One of
these progenitor cell lineages has sustained a neutral polymorphic
marker mutation (for example, loss of O-acetylttransferase activity).
During progenitor cell turnover, lineages are continuously lost to fol-
low-up due to random loss of lineages with replacement by others. The
rate of lineage loss is governed by the symmetric division rate as shown
in A. New marker alleles arise constantly at a normal background
mutation rate. In this example, one progenitor lineage carrying an on-
cogenic mutation (for example, p53 mutation or K-ras activation) arises.
As a result of random genetic drift, this lineage may either attain
dominance (bottom) or be lost to follow-up (top). Thus progenitor cell
populations in a niche and their pool of accumulated mutations contin-
ously change during life as a result of lineage competition. C: normal
unaffected Peutz-Jeghers epithelium demonstrates an expanded progen-
itor compartment. D and E: an expansion of the progenitor pool repre-
sents a cancer-prone state. D depicts a hypothetical series of wild-type
adult colon crypts, whereas E represents a series of Peutz-Jeghers colon
crypts. A greater number of selectable variants is retained over a longer
period of time among the pool of progenitors in Peutz-Jeghers epithel-
ium in E due to protracted clonal evolution of the niche as a result of an
expanded progenitor cell pool. An increase in the pool of selectable
variants accelerates the time to neoplastic transformation, since it in-
creases the likelihood that a fortuitous combination of oncogenic mu-
tations (for example, a biallelic APC mutation) occurs. Mutations (neu-
tral and oncogenic) are sustained under both scenarios at a similar
normal background mutation rate. However, variations in clonal evolu-
tion rate allow mutations to accumulate at different frequencies even
though mutations arise at a similar frequency throughout life. This
lottery-like process is played out at steady-state in each of the ±15
million crypts that constitute the adult human colon. In the example
shown, one lineage carrying an oncogenic mutation has drifted to dom-
nance in the crypt (right). Note that this process is entirely obscure on
routine histopathology, as we have no markers of prospectively tracing
individual stem cell lineages. In this scenario, LKB1 hemizygosity exerts
its oncogenic effects before visible neoplastic transformation, and
Peutz-Jeghers syndrome therefore models an accelerated pretumorigen-

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intestinal tract, PJS patients develop PJS polyps that may serve as a pointer to the cancer-prone condition and adenomatous polyps that are the focus of neoplastic change.

Since clonal evolution of stem cell compartments is obscure on routine histopathology, LKB1 hemizygosity in PJS patients (or in the sporadic setting) is expected to exert its oncogenic effect during “pretumor progression” before visible neoplastic transformation (63). Pretumor progression refers to tumor progression in the absence of phenotypic changes and specifies that stem cells accumulate mutations stochastically from birth in phenotypically normal epithelia; pretumor progression therefore precedes gatekeeper mutation. A protracted clonal evolution scenario resulting from an expanded progenitor compartment accelerates pretumor progression by expanding the pool of selectable variants (Fig. 6). This oncogenic effect of LKB1 hemizygosity on stem cell lineage turnover and the tempo of accumulation of genetic changes in phenotypically unaffected progenitor compartments would similarly pertain to other stem cell niches in the mammalian system, for example, progenitor cell compartments in the epithelial parenchyma of the lung, breast, or exocrine pancreas. Inactivation of the wild-type LKB1 allele may afford selective advantages during visible clonal expansion in terms of, for example, an enhanced migratory potential. Formally testing this concept on the involvement of LKB1 in the regulation of asymmetric stem cell division would require lineage tracing experiments wherein multiple lineages can be traced competing over time in individual crypts in normal and PJS epithelia. Although the mechanism underlying the expanded progenitor compartment remains unclear (whether this may be due either to a cell-autonomous defect in asymmetric stem cell division or due to faulty epithelial-mesenchymal cross-talk), it is clear that LKB1 hemizygosity is not neutral in adult mammalian intestinal epithelial homeostasis. Future studies will address the role of LKB1 in mammalian epithelial stem cell turnover. Demonstration of an expanded stem cell pool in normal PJS epithelium would directly imply a cancer-prone state at normal background mutation rates.

Recent work from our own laboratory has now definitively shown that crypt base columnar cells constitute a stem cell pool of the adult mammalian small intestine and colon (11). Lgr5 represents the first adult mammalian stem cell marker, and it should facilitate research into possible pathways regulating stem cell turnover affected by LKB1. Although evidence exists for a role of LKB1 in asymmetric stem cell division in invertebrate model organisms, so far this has not been reported in the mammalian setting. It is noteworthy in this respect that female PJS patients almost universally display characteristic benign germ cell-derived tumors of the ovaries called SCTATs, as outlined above (135). Meiotic cell division and the expression of a polar body in a primary oocyte represent a particularly remarkable example of asymmetric cell division in the human system, both in terms of asymmetric cell size and cell fate. One interpretation consistent with current data from invertebrate model organisms is that these neoplasms develop due to disrupted asymmetric cell division occurring in meiosis I in female PJS patients.

IX. CONCLUSION

Taken together, current data favor a role for LKB1 in the regulation of cellular polarization through activation of a diverse set of targets such as the AMPK, MARK, or SAD kinases (Fig. 7). It is worth bearing in mind though that Lkb1-deficient mice, remarkably, do not display polarity-related phenotypes during early embryogenesis (134). This suggests that, depending on context, redundant mechanisms may be involved in regulation of polarity establishment. The role of AMPK signaling remains to be more thoroughly characterized. Evidence from a mammalian in vivo model regarding defects in cellular polarization has not been reported, even though data from invertebrate systems and in vitro cell culture models suggest a dominant role for AMPK activation. LKB1 appears to regulate the AMPKα2 isoform, the activity of which is most prominent in liver, heart, and skeletal muscle (Table 2) (4, 111). Moreover, AMPKα2 knockout mice present a metabolic phenotype, unlike the phenotype of the Lkb1+/− mice. However, rescue by the AMPKα1 isoform cannot be excluded in this case. Thus definitive
demonstration for a role of AMPK signaling in mammalian cellular polarization and PJS pathogenesis awaits a conditional mouse model that will allow targeting both AMPK isoforms simultaneously.

The mechanism responsible for the cancer-prone state in PJS patients remains unclear. The majority of studies investigating LKB1 function are performed under LKB1 null conditions. Although this has revealed valuable functional information on the consequences of LKB1 loss, it needs to be underscored that LKB1 hemizygosity is not silent, both with respect to the suppression of polyposis and the suppression of malignant transformation. Thus issues of gene dosage and tissue heterogeneity will need to be taken into account when considering the target(s) of LKB1. It remains unproven that PJS polyps carry an increased potential for malignant transformation compared with the surrounding unaffected epithelium. The molecular mechanism for polyp development must, however, underlie malignant transformation in PJS patients as well. Data from invertebrate model organisms have indicated an important role for LKB1 in the regulation of asymmetric stem cell division. Ectopic symmetric progenitor cell divisions may result in an expanded progenitor cell compartment as observed in unaffected PJS epithelium. This may independently mediate both PJS polyp development and the cancer-prone state in PJS through one molecular mechanism.

The demonstration that the LKB1 locus is affected in tumors from a significant number of sporadic lung cancer patients has increased interest in this enigmatic tumor suppressor protein. Clearly, the importance of research in patients afflicted by rare tumor predisposition syndromes such as PJS is validated by these observations. Current work implicating LKB1 in metabolic adaptation and training endurance further increases interest in the potential use of AMPK isoforms simultaneously.

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