Casein Kinase II in Theileriosis

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East Coast fever is an acute, leukemia-like disease of cattle and Cape buffalo that is endemic in eastern, central, and southern Africa, where the disease causes high mortality and losses in livestock production. It is caused by the parasite *Theileria parva*, which infects bovine lymphocytes and causes cell death by lymphocytolysis. A peculiar and fascinating feature of infection by this parasite is that it only survives in a subset of T lymphocytes, which subsequently become transformed—gaining the ability to infiltrate tissues and survive indefinitely in vitro. Casein kinase II (CKII), a serine-threonine–specific protein kinase that is ubiquitous in eukaryotic organisms, is increased markedly in lymphocyte cell lines from infected cattle, and it has been proposed that this is the molecular basis for the transformation (*1*, *2*). In this week's *Science*, Seldin and Leder (*3*) report on a transgenic mouse model containing a dysregulated CKII gene that provides evidence for the contributory role of CKIIα in oncogenic transformation.

The mammalian-infective stage of *Theileria*, the sporozoites, are injected into vector-attachment sites by a feeding tick. The sporozoites of *T. parva* invade (*4*) B and T lymphocytes as well as null cells, but only survive in and induce proliferation of subpopulations of T cells (*5*). The sporozoites of *Theileria* are nonmotile organisms that are morphologically identical to those of other Apicomplexa, such as *Plasmodium*, and have a surface coat and micronemes and rhopteries (subcellular organelles thought to participate in host cell invasion in Plasmodium spp.). Within minutes of entry into the lymphocyte, the sporozoite induces rapid dissolution of the encapsulating host cell membrane, a process that occurs concurrently with the exocytosis of electron-dense material within the micronemes and rhopteries (*4*, *6*, *7*). Dissolution of the host cell membrane aborts the formation of a parasitophorous vacuole, leaving the sporozoite, now surrounded by a hammock-like array of lymphocyte microtubules, entirely exposed to the elements of the cytosol of the host cell. Within the lymphocyte, the sporozoite differentiates into a multinucleate body, the schizont (which can immortalize lymphocytes in vitro), and undergoes nuclear division ahead of the host cell due to a shortened or absent G_2 phase (*8*). This process ensures synchronous division of the parasite and the host cell, as well as the transfer of schizonts into each daughter cell. In the lymphocyte, the schizont further differentiates into merozoites, which reacquire a surface coat and visible apican organelles, bud off from the schizont, induce lysis of the lymphocyte, and invade erythrocytes. Within the erythrocytes, the merozoites develop into piroplasms, which can then infect ticks.

Zebu cattle, Kenya. *Theileria* infection causes serious mortality in African livestock. [photo courtesy of D. C. Seldin]

In cattle infected with *T. parva*, the proportion of infected lymphoblasts reaches a peak during the second or third week of infection (*9*). Lymphocytolysis of the *T. parva*–infected cells occurs during the third week of infection, and the infected animals usually die of massive pulmonary edema.

The ability of the intralymphocytic schizonts of *T. parva* and *T. annulata* to induce blastogenesis and clonal expansion of T and B cells, respectively, is unique among protozoa. The infected lymphocytes, whether recovered from animals or infected ex vivo, are immortalized and can be propagated indefinitely in vitro (*10*). *Theileria*-infected cells exhibit such telltale signs of transformation as changes in surface epitopes for monoclonal antibodies (*5*), pleiomorphism, and short (16 to 25 hours in vitro) generation times. Above all, the infected lymphocytes infiltrate tissues, forming tumor-like masses in most organs when injected into athymic (*11*) or severe combined immunodeficient (SCID) mice (*12*).

What property of *Theileria* is responsible for this transformation? One possibility, which is supported by the phenotype of the trangenic mouse reported by Seldin and Leder (*3*), is the presence of CKII. The transcription, translation, and activity of bovine CKII are increased in *Theileria*-infected lymphocyte lines (*1*). The activity of CKII also increases progressively in T cell–enriched populations recovered at various time points from *T. parva*–infected cattle (*13*).

Casein kinase II, whose misleading name is derived from its ability to preferentially phosphorylate in vitro acidic proteins such as casein and phosvitin, is distinct from the authentic casein kinases, expressed in the cells of lactating mammary glands (*14*). Studies on CKII and the genes encoding its subunits have implicated this kinase, which is remarkably conserved across large evolutionary distances, in the regulation of various metabolic events, in receptor signaling pathways, and in cellular proliferation and transformation (*14–17*). Because of its many physiological roles in a variety of subcellular locations during most stages of the life of the cell, CKII has been considered an eminence grise of cellular regulation (*14*, *16*).

In most higher eukaryotes CKII is composed of two catalytic (α and α') and two regulatory (β) subunits that combine to form a tetrameric holoenzyme with a possible $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ stoichiometry. However, in *Drosophila* (*18*), *Zea mays* (*19*), and *T. parva* (*2*), only one catalytic subunit has been found. The highest concentrations of CKII have been reported in embryonic tissues, proliferating cells, and in mature neurons (*20*). Although the subcellular localization of CKII by immunohistochemical methods has been a subject of contention, the controversy serves to underscore the enzyme's dynamic distribution, with localization at defined subcellular locations at specific times during the cell cycle. For example, CKII is localized in the nucleus and cytoplasm during the G_0/G_1 transition, in the nucleus in the early phase of G_1, and moves into the cytoplasm for the G_1/S transition (*21*).

Although the functions of CKII in mitogenic signaling pathways are still ill defined, several observations suggest a central role for this enzyme in postreceptor cascades in both the cytoplasmic and nuclear compartments. These include a moderate increase in CKII activity when cells in culture are exposed to serum, hormones, or growth factors (*14–16*). The transition from quiescence to proliferation in adrenocortical cells in culture is also associated with an increase in all subunits of CKII (*22*). Such cellular responses to mitogenic stimulation can be inhibited by microinjection of antibodies to

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CKIIß (*23*). Additionally, IRS1, a principal substrate for the insulin receptor, is phosphorylated by CKII in vitro and, most likely, in intact cells as well (*24*), suggesting that CKII-catalyzed phosphorylation of IRS1 may be a component of the insulin-signaling cascade. In lymphocytes, exposure of T cells to mitogenic stimulation (*25*) and induction of B cell cycle progression by phorbol ester and ionomycin are associated with increases in the enzymatic activity of CKII (*26*).

Among the substrates of CKII in the nucleus are proteins, some of whose mutated forms have been implicated in oncogenic transformation: Myc/Max homo- and heterodimers, Fos, Myb, c-ErbA, and the tumor suppressor protein p53 (*17, 27, 28*). Most of the catalytic subunits form tight associations with nuclear proteins rather than with the CKIIß regulatory subunit (*29*). This finding is supported by reports showing that CKII copurifies with a number of its substrates, including the transcription factor Sp1 (*30*), topoisomerase I (*31*), and yeast topoisomerase II (*32*). In several of the nuclear proteins, mutations or loss of CKII phosphorylation sites is associated with oncogenic conversion (*17*). Another important group of substrates for CKII are the transforming proteins from several DNA tumor viruses, including the large T antigen of SV40, E1A of adenovirus, and E7 of human papilloma virus–type 16 (*17*). Several of these proteins are phosphorylated in vivo at specific CKII sites. Whereas loss of, or mutations affecting, the CKII phosphorylation sites in proto-oncogene products induce oncogenic conversion, such effects render some of the viral proteins transformation-defective (*33*).

Although its presence in the parasite has not been completely ruled out, *T. parva* does not seem to contain a β subunit for CKII. These findings raise the possibility that the parasite CKIIα subunit inserts into the parasite plasma membrane or is secreted into the host cell cytosol at some phase of the cell cycle of *T. parva*. From either of those locations, the parasite enzyme could phosphorylate mammalian substrates without being subject to normal controls and thus alter cell cycle regulation or influence the induction of bovine CKII in a manner similar to that described for c-*jun* (*34*). Heller-Harrison and Czech (*35*) have recently shown a two- to fivefold enhancement of endogenous CKII activity, depending on whether one or both genes encoding the catalytic or regulatory subunits of human CKII had been transfected into COS-1 cells. Therefore, CKII may be an important element in a signal-transducing pathway activated by *Theileria* in lymphocytes, giving rise to transformation when its regulation becomes aberrant (*1, 2*).

The transgenic CKIIα mouse model reported by Seldin and Leder (*3*) provides evidence of such a role for CKIIα. In the cells of the transgenic mice, the transgenic CKIIα transcripts represent a notable proportion of the total CKIIα mRNA. Although there was no detectable increase in the total amount of CKIIα protein or in the phosphotransferase activity of the enzyme, the transgenic animals exhibited a high predisposition to develop lymphoma. Coexpression of the CKIIα transgene with c-*myc*, a proto-oncogene whose product is a substrate of CKII in vivo (*36*), results in an alarmingly rapid development of murine perinatal leukemia associated with disruption of lymphoid cell functions.

There are interesting similarities between the transgenic mouse model, in which deregulated expression of CKIIα contributes significantly to the onset of lymphoma, and theileriosis, a disease in which CKII is induced by parasite factors. The transgenic murine lymphocytes are clonal populations of cells that require the activity of CKII to continue dividing (*3*). *Theileria*-induced transformation is also reversible because administration of antitheilerial drugs will kill the parasite, and the cells stop dividing and usually die, unless they are rescued by inclusion of mitogens in the growth medium. It should also be possible to arrest *Theileria*-induced host cell growth by administration of antisense CKIIα oligonucleotides as reported for neuroblastoma cells (*20*) and mouse CKIIα transgenic lymphocytes (*3*).

The tissue-infiltrating behavior of the CKII transgenic lymphocytes, described by Seldin and Leder (*3*), is also a pathophysiological feature of acute, fatal *T. parva* infection in cattle. Such cells are particularly notable in the kidney where thick sheets of infected and activated lymphocytes infiltrate the connective tissues between the tubules and around glomeruli (*37*). Similar disorganized masses of lymphocytes occur around hepatic interlobular vessels. Within the circulatory system, such cells aggregate in venule and capillary vessels, sometimes producing micro-infarcts, primarily in the kidney and brain.

The implicit assumption in reports of high levels of CKII enzymatic activity in a variety of human neoplasms is that such high levels of the enzyme are a manifestation of cellular proliferation (*14*) or of dysregulated expression. However, as shown by Seldin and Leder (*3*) and by others, neoplastic transformation induced by dysregulated expression of a critical regulatory element in a signal-transducing, or other, cascade of the cell, is not necessarily associated with elevated concentrations of the particular element. It will be of interest to determine whether the chromosomal translocations reported in human lymphoid and other neoplasms, which involve loci-bearing CKII genes, are associated with dysregulated expression of the genes encoding any of the three subunits of human CKII or the subunits of the type 2A protein phosphatases modulating the activities of the substrates of CKII.

The reported tendency of oncogenes to be cell type–specific, favoring the formation of specific tumor types, also applies to *Theileria*. If an oncogene is virally transduced, selectivity occurs not only at the level of the membrane receptor, but also because the cell types giving rise to such neoplasms must provide a facilitatory biochemical environment for the action of the particular oncogenes. Although *T. parva* enters a variety of lymphoid cell types, the parasite transforms most efficiently subpopulations of T cells. Indeed, among the important determinants of the restricted range of cell types that *Theileria* sporozoites can invade and survive in are perhaps the expression of major histocompatibility complex class I molecules–β_2-microglobulin and the rate of host membrane dissolution by the parasite, relative to that of formation of functional lysosomes by the host cell. A cell fulfilling these criteria must also provide a suitable biochemical context for the dysregulated molecule to activate downstream steps in a cascade that induces cellular transformation. There may be no better way to dissect mitogenic pathways of certain mammalian cells than that of finding and characterizing the roles of the essential elements usurped or selectively activated by oncogenic viruses and other transforming organisms, such as *Theileria*, which have had a long history of residence within the cells of their mammalian hosts.

References and Notes

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Programmed Cell Death in Bacterial Populations

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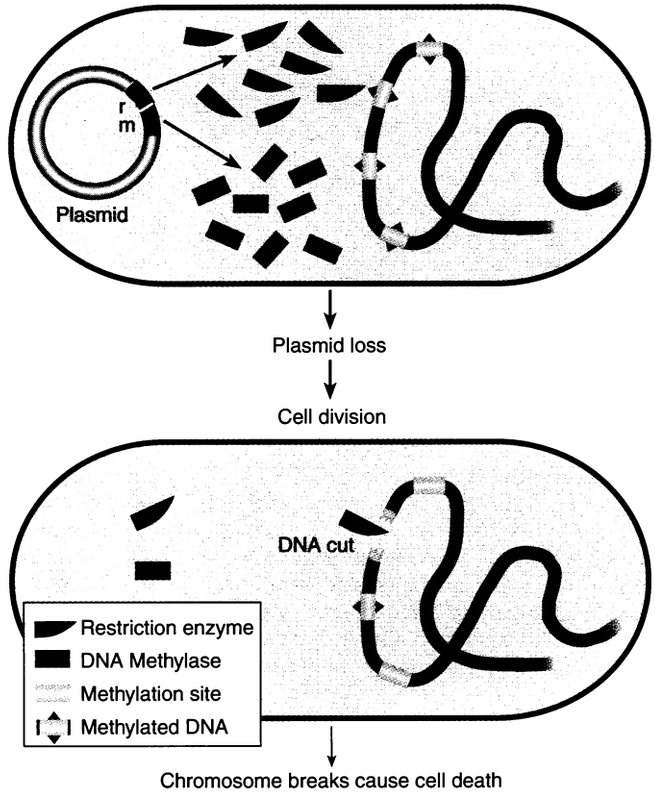
Multicellular organisms benefit not only from the death of competitors, but often, quite dramatically, from the programmed death of specific subpopulations of their own cells. Popular opinion to the contrary, programmed cell death is also alive and well in the microbial world. A striking report by Naito *et al.* in this issue of *Science* (*1*) is a case in point.

If bacteria are to profit from the death of their own kind, they must be heterogeneous. Indeed, temporal and positional variation of cell type in clonal populations is the norm, not the exception. Switching among genetic and epigenetic states in response to cell density, nutrient supply, substratum surface, plasmid burden, incident radiation, viral infection, or the passage of time accounts for such effects as the variety of colonial forms and, among pathogens, the defeat of the host's immune response (*2*, *3*). Unexpected differentiated states have been found even in thoroughly studied *Escherichia coli* (*4*). It has also been proposed that starvation induces in this bacterium the appearance of a hypermutable state in a moribund subpopulation (*5*). Moribund subpopulations, whose members are not recoverable as colony-formers, may readily escape notice. They are associated not only with mutability, but also with the carriage of another major source of bacterial adaptability, plasmids.

A growing number of large plasmids have been shown to program the death of plasmid-free segregants (*6*). This strategy prevents the survival of bacterial mutants disabled for plasmid retention that would otherwise overgrow the plasmid carriers. The set of genes carried by a plasmid that is responsible for the lethal consequences of plasmid withdrawal can be viewed as an "addiction module"; it renders the bacterial host addicted to the continued presence of the "dispensable" genetic element it harbors.

A plasmid addiction module makes a simple time bomb: the charge, a stable toxin; the timer, a labile antidote. Detonation occurs when the ratio of antidote to toxin becomes too low. In the plasmid-free cell, neither antidote nor toxin is replenished and the antidote is eliminated more rapidly than the toxin, leaving the latter to exert its lethal potential. In some cases, dilution alone may suffice to eliminate antidote function before dilution has rendered the toxin innocuous. Several familiar type II restriction enzymes and their cognate methylases are plasmid-encoded and all of them are toxin-antidote pairs, the DNA methylase offering protection from endonucleolytic attack by the separate restriction enzyme. It is easy to see how the progeny of a cell that managed to rid itself of a r^+m^+ plasmid (one that carries restriction-modification genes) might retain insufficient methylase to protect itself from the remaining restriction enzyme and so be killed (see figure). Enhancement of the apparent stability of a plasmid that encodes a type II restriction enzyme and its cognate methylase is precisely what Naito *et al.* (*1*) observed with both Pae R7 and Eco RI rm genes. Examples will surely multiply.

Toxin lethality is normally held in check in a variety of ways (*6*). DNA methylases modify restriction enzyme targets (specific DNA sequences) so as to render them unassailable. In the family of toxin-antidote pairs to which toxins Gef and Hok belong, binding of antisense RNA to a long-lived precursor of the messenger RNA that encodes the toxin prevents toxin synthesis. In



Plasmid loss avenged. Bacterial death programmed by a plasmid-encoded restriction-modification system.

ILLUSTRATION: C. FABER SMITH

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