tween female-determining genes on the X chromosome and male-determining genes on the autosomes" was a formalism to account for the reciprocal influences of X chromosome and autosome dose on sexual development. We now know that the specific X-linked female determiners exist and that at least one, *sis-b*, is a conventional (protein encoding) gene. The male determiners remain a formalism and may never be more than that.

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10. Mutant $In(1)ac^3$ sc^{10-1} flies are nearly devoid of bristles because of a rearrangement affecting transcription unit T5 and a point mutation within T4 (*4, 6*). With the exception of an occasional sex-comb tooth, the $T4^+$ transgenes failed to restore any of the macrochaetae that develop normally in $In(1)ac^3$ individuals. In some cases a small fraction of the microchaetae eliminated by the ac^3 (T5) rearrangement were restored.
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Enhancement of HIV-1 Cytocidal Effects in $CD4^+$ Lymphocytes by the AIDS-Associated Mycoplasma

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Coinfection with *Mycoplasma fermentans* (incognitus strain) enhances the ability of human immunodeficiency virus type–1 (HIV-1) to induce cytopathic effects on human T lymphocytes in vitro. Syncytium formation of HIV-infected T cells was essentially eliminated in the presence of *M. fermentans* (incognitus strain), despite prominent cell death. However, replication and production of HIV-1 particles continued during the coinfection. Furthermore, the supernatant from cultures coinfected with HIV-1 and the mycoplasma contained a factor that inhibited the standard reverse transcriptase enzyme assay. The modification of the biological properties of HIV-1 by coinfection with mycoplasma may be involved in the pathogenesis of acquired immunodeficiency syndrome (AIDS).

HIV-1 INFECTION OF EXPERIMENTAL animals, including chimpanzees, has not produced any specific illnesses other than transient lymphadenopathy despite evidence of more than 7 years of persistent viremia. Furthermore, HIV-infected patients often show a wide variation in times of disease incubation and speed of disease progression. It is not known whether any specific infectious agent other than HIV can be responsible for the complex pathogenesis often seen in this disease. One such candidate, initially reported as a virus or virus-like infectious agent, is the mycoplasma *M. fermentans* (incognitus strain) (*1–4*). The organism causes a fatal systemic infection in primates (*5*) and induces necrotizing lesions in the lymph nodes, spleens,

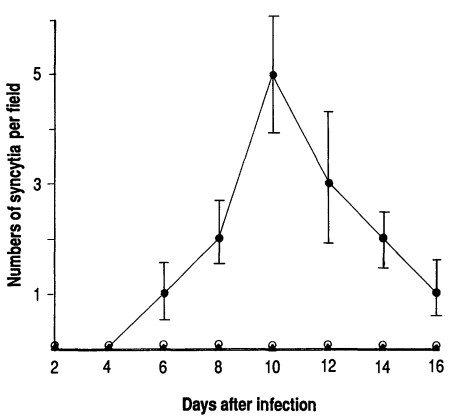

Fig. 1. Inhibition of HIV-1–induced syncytium formation by *M. fermentans* (incognitus strain). We infected A3.01 cells (5×10^7) with (▲) HIV-1 (1×10^5 infectious units) and incognitus strain (1×10^3 infectious units), (●) HIV-1, or (○) incognitus strain. Cells in each culture were incubated at 37°C for 2 hours and then washed once with RPMI 1640 medium. The infectious titer of HIV-1 was previously determined by exposing A3.01 cells to tenfold serial dilutions of HIV-1 culture stock for 2 hours at 37°C. The highest dilution in which the presence of RT activity could be detected after 14 days in culture represented one infectious unit. We grew the incognitus strain in modified SP-4 media and filter-cloned it three times from a single colony on agar plates (*3*). The organisms were washed once and resuspended in RPMI 1640. The titer of incognitus strain after infection of NIH 3T3 cells was determined by an antigen dot blot assay (*3*). The cell cultures were maintained with RPMI 1640 supplemented with 10% fetal bovine serum. Results are the average of the number of syncytia per field (×200) of ten fields examined per culture. The error bars indicate standard deviation of the mean.

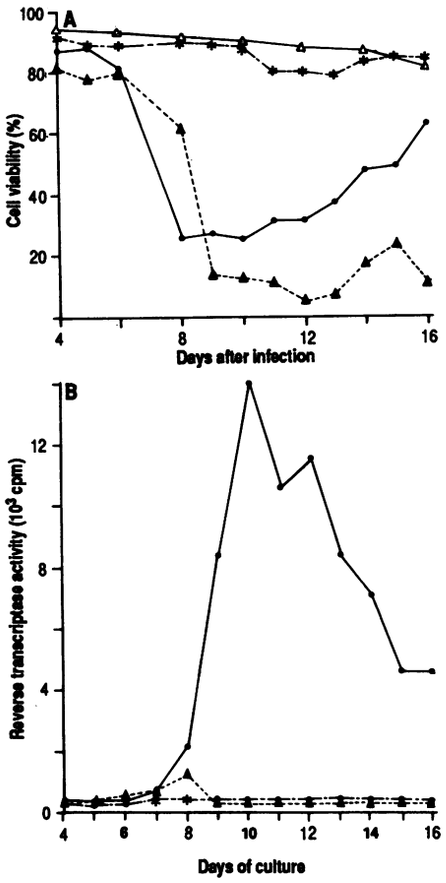

Fig. 2. Augmentation of cytocidal effect and inhibition of RT activity in HIV-1–infected A3.01 cell cultures by *M. fermentans* (incognitus strain). A3.01 cells were cultured after (●) infection by HIV-1, (▲) infection by HIV-1 and incognitus strain, (△) infection by incognitus strain, or (*) no treatment. Each point on each graph is the average of the results of three independent cultures (**A**) We determined cell viability with the Trypan blue exclusion test with a total of 200 cells counted for each time point. (**B**) We tested daily samples of culture supernatants with the standard RT enzyme assay using the incorporation of tritiated triphosphate nucleotides (*21*). Conditions of HIV-1 and mycoplasma infections were the same as described in the legend to Fig. 1. The culture infected by mycoplasma alone [indicated by △ in (A)] also had no detectable RT activity (data not shown).

livers, adrenal glands, hearts, or brains of previously healthy people that result in an acute, fatal disease (*6*). Many patients with AIDS also have a systemic infection with this microbe (*2, 7*). We examined the effects of the AIDS-associated mycoplasma, incognitus strain, on HIV-1 infection of a $CD4^+$ human T lymphocyte cell line, designated previously as A3.01 (*8, 9*).

Normally, HIV-1 infection of human T lymphocytes in vitro produces pronounced cytopathic effects (CPE) with the release of newly replicated virus (*10*). The formation of large multinucleated cells, termed syncytia, and high levels of reverse transcriptase (RT) activity is a characteristic feature of HIV-1 infection in vitro (*11*). Large numbers of syncytia formed when HIV-1 alone infected A3.01 cells, but syncytium formation disappeared in A3.01 cells simultaneously infected with HIV-1 and incognitus strain (Fig. 1) despite clear evidence of a cytocidal effect. The incognitus strain significantly enhanced the cytocidal effects of HIV-1 infection in A3.01 cells (Fig. 2A). Furthermore, populations of cells that had been infected by HIV-1 alone gradually recovered from the initial cytocidal effect and remained persistently infected (*12*). In contrast, A3.01 cells infected by both HIV-1 and incognitus strain died. In this study, incognitus strain infection alone did not produce detectable cytotoxicity. As expected, culture supernatants from A3.01 cells infected with HIV-1 had clear RT activity. However, samples from the coinfected cell culture showed little or no RT activity (Fig. 2B).

Despite the absence of RT activity, virus-specific protein synthesis and assembly was occurring. The coinfected cell culture produced HIV-1–specific p24-p25 as rapidly as the culture infected by HIV-1 alone (Fig. 3A). Electron microscopy of coinfected cells showed typical HIV virions (Fig. 3B). The assembled virions were infectious. Supernatant from the coinfected culture, which showed no detectable RT activity, was tenfold serially diluted and incubated with fresh A3.01 cells. We found comparable infectious units of HIV-1 (10^5 per milliliter) to be produced in the supernatants after infection of cell cultures either by HIV-1 alone or by both HIV-1 and incognitus strain (*13*).

To test if substances in cultures infected by incognitus strain directly affected the RT enzyme assay, culture supernatant from A3.01 cells coinfected with HIV-1 and incognitus strain was mixed with the culture supernatant containing HIV with known RT activity. Over 90% of the RT activity was inhibited when less than a third of the active supernatant was replaced by culture supernatants containing both HIV-1 and incognitus strain (Fig. 4). Enzyme inhibition occurred immediately, and prior incubation of the mixture of culture supernatants was not required. We observed a comparable degree of inhibition when we used culture supernatant from A3.01 cells infected with only incognitus strain in the inhibition assay. Thus, the results can be best explained by the presence of some mycoplasma product or products in the assay lysate which directly interfered with the RT assay. Some mycoplasmas have recently been found to produce highly active nucleases (*14*), which could potentially be involved.

The masking effect of HIV RT activity may not be unique to incognitus strain. Suppression of HIV RT has recently been reported in *M. hyorhinis*–contaminated lymphocyte cultures (*15*). But in contrast to the results in this report, the HIV-1–infected cultures contaminated by the swine mycoplasma still formed prominent syncytial cells. Our study indicates that syncytium formation and the actual cytocidal effect can be separate events. Our findings support the

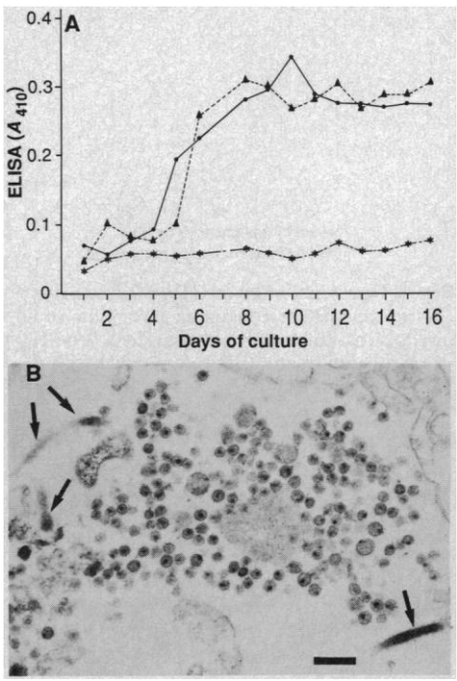

Fig. 3. Continued viral production of HIV-1 in A3.01 cells coinfected with HIV-1 and *M. fermentans* (incognitus strain). (**A**) Culture supernatant (100 μl) was tested for the presence of viral antigen (HIV-1 antigen assay kit, Integrated Diagnostics, Gaithersburg, Maryland). The assay kit uses an enzyme-linked immunosorbent assay (ELISA) technique, and the procedures performed in this study were in strict accordance to the instructions supplied with the kit. The negative control (phosphate-buffered saline) had an absorbance (A_{410}) reading of less than 0.1 at 410 nm. Each point on the graph is the average of the results of three independent cultures. (●) A3.01 + HIV-1, (▲) A3.01 + HIV-1 + incognitus strain, (*) A3.01. (**B**) Electron micrograph of a cell culture infected simultaneously with both HIV-1 and incognitus strain. Numerous viral particles are seen in this culture with lytic cells. Occasional electron-dense forms of incognitus strain (arrows) can also be seen. Bar = 400 nm.

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earlier reports (*16, 17*) that state that the formation of syncytial cells is not a necessary prerequisite for proliferation of HIV-1.

It has recently been shown that nontoxic doses of the antibiotic tetracycline may significantly reduce the cytocidal effects of HIV-1 (*18*). The tetracycline-treated cultures continued to produce a high titer of HIV-1. The authors suggested that a prokaryotic agent, most likely a mycoplasma, was involved with the cytocidal effect observed in the HIV-infected cultures. Indeed, additional study and characterization from their laboratory has confirmed that the hidden agent in the cultures is a mycoplasma (*19*).

Researchers from Japan have reported that just the antigens of killed mycoplasma (*Acholeplasma laidlawii*) could stimulate HIV-1 production (p24 antigen and infectious particles) in HIV-1–infected cells (*20*). In our study, approximately equivalent amounts of HIV antigen or infectious particles were produced in HIV-infected or HIV and incognitus strain–infected cultures despite significant differences in the numbers of viable cells. Thus, more HIV-1 may actually have been produced per individual cell in the coinfected culture; this finding is similar to the findings of the Japanese researchers.

AIDS patients can be infected with a number of pathogenic microbes and frequently are systemically infected with the incognitus strain (*2, 7*). Thus, the observation that coinfection by incognitus strain profoundly enhances cytocidal effects of HIV-1 infection in vitro may have clinical implications.

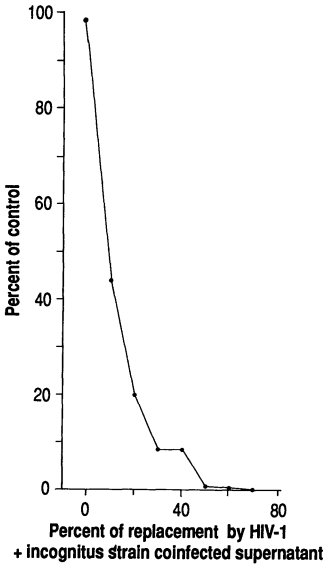

Fig. 4. Direct inhibition of HIV-1 RT activity by culture supernatant containing incognitus strain mycoplasma. RT activity was assayed (*21*) on 100 μl of reaction mixture, 50 μl of which was the "reaction cocktail." For the remaining 50 μl, varying proportions of culture supernatant with a known amount of HIV;1 RT activity were replaced by supernatant from a culture coinfected for 10 days with HIV-1 and incognitus strain (Fig. 2). The degree of inhibition, expressed as "percent of control," was obtained by comparing the RT activity detected in each sample with that from a control sample containing RPMI 1640 medium instead of coinfected culture supernatant. Each point on the graph is the average of the results of three independent experiments. At 0% replacement, sample variability was 2.4%.

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A Model for the Adjustment of the Mitotic Clock by Cyclin and MPF Levels

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A mathematical model of cell cycle progression is presented, which integrates recent biochemical information on the interaction of the maturation promotion factor (MPF) and cyclin. The model retrieves the dynamics observed in early embryos and explains how multiple cycles of MPF activity can be produced and how the internal clock that determines durations and number of cycles can be adjusted by modulating the rate of change in MPF or cyclin concentrations. Experiments are suggested for verifying the role of MPF activity in determining the length of the somatic cell cycle.

RECENTLY IT HAS BEEN SUGGESTED that a single biochemical mechanism underlies the cell cycle progression in all eukaryotic organisms (*1, 2*). The basic mechanism involves activation and inactivation of the maturation promoting factor (MPF) by a protein called cyclin. During the interphase, cyclin accumulates until the rate of MPF activation by cyclin exceeds the rate of inactivation of MPF by MPF-inactivase, the concentration of which is assumed to be constant. As a consequence, active MPF accumulates, which leads to a series of modifications of other mitotic substrates. The activation of MPF also induces the activation of cyclin degradation, and cyclin disappearance results in MPF destabilization by MPF-inactivase. The interphase structures are then reestablished, and cyclin begins reaccumulating to initiate the next cycle. This model is generally consistent with experimental data (*1*).

In early embryonic development, MPF activity serves as an internal clock that suffices to send the cell into mitosis (*1, 3*). In the present work we examine the regulation of this clock using a mathematical model for the MPF and cyclin reactions. Underlying our model is the principle of parsimony: by elucidating the minimal assumptions needed to retrieve the observed dynamics, we determine whether thresholds, such as the one postulated for signaling cyclin degradation (*4*), or time delays, thought to be missing in the present biochemical model (*1*), are in-

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