HeLa cells by infection with a MOI = 1. Infected cells were incubated for 8 hours at 37°C, washed with phosphate-buffered saline (PBS), and lysed by freeze-thawing in 1.5 ml of PBS. Mice were infected by intraperitoneal injection with recombinant poliovirus (100 μl of 2 × 10^8 pfu per milliliter of stock) or mock infected with PBS. PVR-transgenic mice received two identical injections separated by a period of 30 days. Four weeks later, mice were bled and their sera tested for the presence of specific antibodies. Purified HIV-1 Gag and Nef (produced in *Escherichia coli*, 3 μg each) or poliovirus-infected HeLa cell lysate (100 μg) was subjected to 10% SDS-PAGE in a single wide lane and transferred to nitrocellulose. Sera (diluted 1:200) from immunized animals were loaded into independent lanes of a multislot apparatus (Mini-protean II, MultiScreen apparatus, Bio-Rad). Antibodies reacting with the HIV-1 and poliovirus proteins were visualized with secondary antibodies to mouse Ig (anti–mouse Ig) (Amersham). ELISA was performed as described (*16*) with anti-mouse Ig secondary antibodies (Amersham).

13. A cynomolgus monkey was infected by direct deposition of single doses of 10^7 pfu of mo-hnef in 2 ml of saline. Topical rectal administration was carried out with soft, lubricated pediatric nasogastric tubes. Blood was collected weekly from femoral vessels, and the serum was separated. Rectal washings were obtained atraumatically by flushing 2 ml of PBS into the rectum and collecting the fluid with the aid of lubricated pediatric nasogastric tubes. Examination of rectal and serum antibodies was performed by ELISA (*16*). Plates coated with antigen (recombinant Nef at 5 μg/ml) were incubated with doubling dilutions of test samples. Bound antibody was detected by incubation with antibodies to human Ig and IgA secondary antibodies conjugated to horseradish peroxidase (Amersham and Zymed Labs), followed by determination of enzymatic activity with ABTS tablets (Boehringer). The absorbance was measured at 405 nm. Results are expressed as the reciprocal of the lowest dilution that gave an absorbance of 0.1 to 0.15 U above the background. Controls included assay plates coated with bovine serum albumin and preimmune samples. The reproducibility of the ELISA after three repeated assays of the same sample of serum or rectal washings was within one dilution.
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Prophylactic Vaccines, Risk Behavior Change, and the Probability of Eradicating HIV in San Francisco

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Theory is linked with data to assess the probability of eradicating human immunodeficiency virus (HIV) in San Francisco through the use of prophylactic vaccines. The necessary vaccine efficacy levels and population coverage levels for eradication are quantified. The likely impact of risk behavior changes on vaccination campaigns is assessed. The results show it is unlikely that vaccines will be able to eradicate HIV in San Francisco unless they are combined with considerable reductions in risk behaviors. Furthermore, if risk behavior increases as the result of a vaccination campaign, then vaccination could result in a perverse outcome by increasing the severity of the epidemic.

Mass vaccination campaigns against HIV will be initiated after vaccine efficacy has been established by phase III clinical trials. However, before the vaccination campaigns begin it is important to determine the potential epidemiological impact of the vaccines. Previously we have formulated and analyzed a transmission dynamic model of HIV in order to develop a quantitative framework for assessing the utility of prophylactic vaccines for epidemic control (that is, for eradication and for noneradicating control) (*1*). Here we link this theoretical work with a specific data set to assess whether it will be possible to eradicate HIV in San Francisco through the use of prophylactic vaccines. Data from the San Francisco Young Mens Health Study (SFYMHS), which is an HIV transmission study of young gay men, were used in the analysis. Specifically for San Francisco, three questions were addressed: (i) What proportion of the young gay community would have to be vaccinated in order to eradicate HIV, (ii) how effective would the vaccines have to be to ensure epidemic eradication, and (iii) what effects could changes in sexual risk behavior have on the impact of mass vaccination programs?

Vaccine efficacy is generally calculated with clinical trial data and a standard definition of efficacy, where efficacy is defined as 1 − relative risk. Incidence rates determine the magnitude of the relative risk between the vaccinated and the placebo groups; hence, the calculated efficacy level will depend on the length of the clinical trial (that is, efficacy is time-dependent) (*2–4*). Furthermore, the standard definition of vaccine efficacy does not provide a means for including the specific mechanisms of vaccine failure. Hence, previously we formulated a new measure of efficacy (that we named vaccine impact) so that we could (i) examine the effects of specific mechanisms of vaccine failure and (ii) derive a time-independent summary measure of vaccine imperfection (that is, efficacy) that could be used to calculate the critical vaccination coverage required for epidemic eradication (*1*). This new measure of efficacy was derived while the effects of mass vaccination programs were modeled on the transmission dynamics of HIV in a gay community. The transmission model consisted of four ordinary differential equations; the model structure is described elsewhere (*1*). Our model included three mechanisms of vaccine failure: take (the fraction of vaccine recipients in whom the vaccine has any immunological effect at all), degree (the degree of reduction in susceptibility per sexual partnership for those in whom the vaccine takes), and duration (the duration of vaccine-induced immunity) (*1*). We named our efficacy measure the impact of the vaccine (ϕ) (therefore efficacy and impact are synonyms) (*1*); throughout this

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report the term efficacy will be used instead of impact. Efficacy can be presented either as a fraction or as a percentage; therefore, if the impact (ϕ) of a vaccine is 0.6, then the efficacy can be described either as 0.6 or 60%. A definition of ϕ is given in Eq. 1; the derivation of ϕ is described elsewhere (*1*). It may be seen that the value of ϕ is determined by the product of three components: the take of the vaccine, the degree of the vaccine, and the fraction of individuals who cease sexual activity before the vaccine-induced protection wanes.

$$\phi = \epsilon\psi \frac{\mu}{\mu + \omega} \quad (1)$$

where ϵ is the take (the fraction of vaccine recipients in whom the vaccine has any immunological effect at all), ψ is the degree (the degree of reduction in susceptibility per sexual partnership for those in whom the vaccine takes), $1/\omega$ is the average duration of vaccine-induced immunity, $1/\mu$ is the average length of the sexual life-span, and $\mu/(\mu + \omega)$ is the fraction of individuals who cease sexual activity before the vaccine-induced protection wanes.

The efficacy measure (ϕ) has three important implications. Firstly, ϕ provides a quantitative measure for comparing different prophylactic vaccines and deciding which vaccine will provide the most effective epidemic control. Secondly, it can be seen from the definition of ϕ that the efficacy even of a vaccine that appears promising may be low because different mechanisms of vaccine failure compound in a multiplicative manner. Thirdly, ϕ can then be used to calculate the critical vaccination coverage (p_c) required to eradicate an HIV epidemic:

$$p_c = \frac{1}{\phi}[1 - (1/R_0)] \quad (2)$$

where ϕ is the vaccine impact (efficacy), and R_0 is the basic reproductive rate of HIV. R_0 is the average number of secondary infections that occur when one infectious individual is introduced into a population of susceptibles (*5*, *6*).

Here we apply these previously derived theoretical results to a specific data set (the SFYMHS) in order to assess the probability for the eradication of HIV in San Francisco. The probability of eradication depends on the severity of the epidemic; hence SFYMHS data were used to assess the severity of the current HIV epidemic in San Francisco. The SFYMHS is a large multistage probability sample of young (18- to 29-year-old) gay men conducted in 1993 in San Francisco (*7*). As a consequence of the sampling design, the SFYMHS is a population-based estimate of HIV infection and sexual behaviors; the methodology of the survey and preliminary results are described elsewhere (*7*). Sexual risk behavior data (both the reported number of receptive anal sex partners and condom usage) were used to estimate the basic reproductive rate (R_0) of HIV. R_0 is an aggregate parameter that quantifies the severity of an epidemic; if R_0 is greater than unity an epidemic can occur, and if R_0 is less than unity an epidemic will not occur. Using the SFYMHS data, we obtained a lower bound estimate ($R_0 = 2$) and an upper bound estimate ($R_0 = 5$) (*8*). These estimates of R_0 were used to calculate the doubling time of the HIV epidemic (Fig. 1). The doubling time lies somewhere between 2 (using the upper bound estimate of R_0) and 7 years (using the lower bound estimate of R_0). Hence the epidemic is fairly severe, a result that is corroborated by the fact that the seroprevalence level in young gay men in San Francisco has already reached 18% (*7*).

Upper and lower bound estimates of R_0 and Eq. 2 were used to calculate the proportion of the young gay community in San Francisco that would have to be vaccinated [by vaccines of varying levels of efficacy (ϕ)] in order to eradicate HIV. The results show that vaccines with low efficacy or vaccines that are administered in a gay community affected by a severe epidemic (high R_0) will not eradicate the epidemic (Fig. 2A). Specifically, it can be seen for San Francisco that if the lower bound estimate of R_0 reflects the true value of R_0, then the minimum efficacy of the vaccine has to be at least 50% to ensure epidemic eradication. An example of a vaccine that would

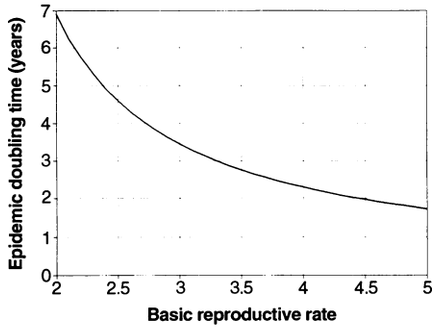

Fig. 1. Doubling time of the HIV epidemic in San Francisco in young gay men; data are from the SFYMHS.

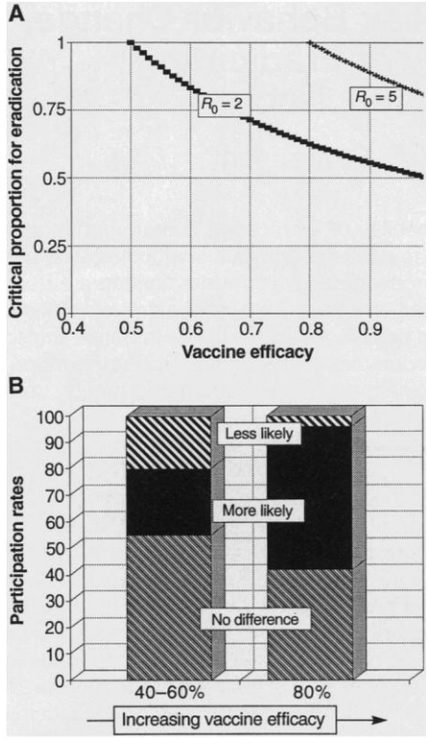

Fig. 2. (**A**) Relation between the critical proportion for eradication and the efficacy level (ϕ) for the upper bound ($R_0 = 5$) and lower bound ($R_0 = 2$) estimate of the basic reproductive rate; data are from the SFYMHS. Efficacy is plotted as a fraction and not as a percentage. (**B**) Relation between vaccine efficacy and potential participation rates in a phase III HIV prophylactic vaccine trial in young gay men in San Francisco; data are from the SFYMHS (*11*).

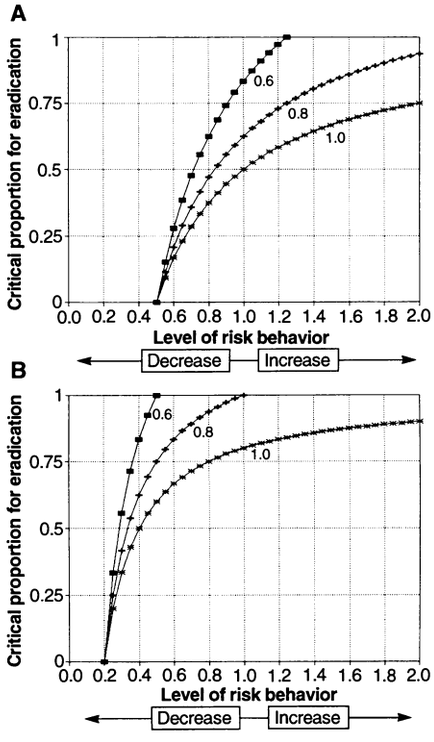

Fig. 3. Relations between the critical proportion that needs to be vaccinated for HIV epidemic eradication and the level of risk behavior; three vaccines are plotted [efficacy (ϕ): 0.6 (60%), 0.8 (80%), 1.0 (100%)]. Level of risk behavior is standardized to the level of risk behavior at the beginning of the mass vaccination campaign. (**A**) Data are from the SFYMHS lower bound estimate of the basic reproductive rate ($R_0 = 2$). (**B**) Data are from the SFYMHS upper bound estimate of the basic reproductive rate ($R_0 = 5$).

have an efficacy of 50% would be a vaccine that would take in 85% of the vaccinated individuals, reduce susceptibility to infection by 95%, and have a duration of immunity that waned with a half-life of 35 years. However, if the upper bound estimate of R_0 reflects the true value of R_0, then the minimum efficacy of the vaccine has to be at least 80% to ensure epidemic eradication. It should be noted that at both these minimum efficacy levels, the vaccination coverage levels would have to be 100%; at lower coverage levels, the efficacy of the vaccines would have to be higher (Fig. 2A).

Participants in the SFYMHS were asked questions regarding the likelihood of their participating in a phase III trial for vaccines of different levels of efficacy. The results show that at lower levels of efficacy, young gay men are less likely to participate in a phase III trial (Fig. 2B). These participation rates may reflect the attainable coverage levels during a mass vaccination campaign; if so, the data shown in Fig. 2B are very discouraging when compared with the results in Fig. 2A, which demonstrate that as efficacy levels decrease, vaccination coverage levels have to increase. The results in Fig. 2B demonstrate that as efficacy levels decrease, participation rates are likely also to decrease. Hence, unless very high efficacy vaccines become available, it may be impossible to eradicate the epidemic because of low participation rates.

Figure 2A illustrates the relation between coverage levels and efficacy levels for the epidemic in San Francisco, under the assumption that risk behavior change does not occur. However, when a mass vaccination campaign is introduced, it is possible that risk behaviors will change; consequently, the effects that changes in sexual risk behavior could have on the impact of mass vaccination programs in San Francisco were explored. Figure 3 shows the coverage levels that are necessary for epidemic eradication in San Francisco for three vaccine efficacy levels (60%, 80%, and 100%); the effect of no change and of increases and decreases in risk behavior are shown for $R_0 = 2$ (Fig. 3A) and for $R_0 = 5$ (Fig. 3B). The variable (βc) which we use to specify the relative level of risk behavior in Fig. 3 is a product of two quantities: the average transmission efficiency of HIV per sexual partnership (β) (which reflects the degree of condom use) and the average number of receptive anal sex partners experienced per unit time (c) (9). This relative level of risk behavior was standardized to the level of risk behavior at the beginning of the vaccination campaign; therefore, in Fig. 3, the relative level of the risk behavior at the beginning of the vaccination campaign was 1. The effects of a completely efficacious vaccine were analyzed, although it is extremely unlikely that such a vaccine will be developed (a vaccine that has an efficacy of 100% would work in everybody, provide complete protection during every encounter, and the vaccine-induced immunity would last throughout the entire sexual life-span).

In the absence of risk behavior change, if the true value of R_0 is 2, all three vaccines could eradicate HIV in San Francisco (Fig. 3A); the necessary eradication coverage levels for each vaccine can be read from the y axis when the level of risk behavior (on the x axis) is equal to 1. However, if the true value of R_0 is 5, then it can be seen from Fig. 3B that in the absence of risk behavior change, a 60% efficacious vaccine would be inadequate for epidemic eradication in San Francisco. Even a vaccine with an efficacy level of 80% would require 100% coverage levels for eradication, and a completely efficacious vaccine would require 80% coverage levels (Fig. 3B).

Figure 3 illustrates how decreases in risk behavior could interact with mass vaccination campaigns. If risk behavior decreases, then all three vaccines may eradicate the epidemic, and eradication is possible at lower coverage levels. Even if the true value of R_0 is 5, it could become possible to eradicate the epidemic with a 60% efficacious vaccine if risk behavior could be halved (Fig. 3B). Furthermore, Fig. 3 shows that reductions in risk behavior alone, without a mass vaccination campaign, could eradicate HIV; in Fig. 3A ($R_0 = 2$), risk behavior levels would have to decrease by 50% of their initial value, and in Fig. 3B ($R_0 = 5$) risk behavior levels would have to decrease by 80% of their initial value.

Figure 3 also reveals how increases in risk behavior could affect the impact of a mass vaccination campaign. If the true value of R_0 is 2, then Fig. 3A shows that if the vaccine efficacy level is very high (80% or 100%), the levels of risk behavior can double, but the epidemic can still be eradicated if coverage levels are increased. However, if the vaccine efficacy level is only 60% and the levels of risk behavior increase by only a slight amount (>1.2), the epidemic can no longer be eradicated even with 100% coverage. If the true value of R_0 is 5, then Fig. 3B shows that if risk behavior increases by any degree, a vaccine that is 80% efficacious could no longer eradicate the epidemic, and only the completely efficacious vaccine could result in eradication.

It is also possible that if risk behavior increased as a consequence of a mass vaccination campaign, then not only may it become impossible to eradicate the HIV epidemic, but mass vaccination may have the perverse outcome of increasing the severity of the epidemic. The probability of such a perverse outcome depends on three factors: the degree to which the risk behavior is increased (D) (that is, D equals the new level of risk behavior divided by the initial level of risk behavior), the efficacy (ϕ) of the vaccine used, and the achieved coverage levels (C). If the inequality shown in Eq. 3 is satisfied, then a mass vaccination campaign could do more harm than good.

$$D > \frac{1}{(1 - \phi C)} \quad (3)$$

For example, a mass vaccination campaign could increase the severity of the epidemic if it was only possible to achieve 50% coverage levels for a 60% effective vaccine and the level of the risk behavior increased by a factor of 1.4.

In this initial analysis of mass vaccination campaigns for HIV eradication, a simple homogeneous model has been used to estimate the coverage levels that are necessary for eradication. Therefore, it has been assumed that (on average) all men are equal with respect to their level of sexual activity and that vaccination is uniformly applied. The issue of heterogeneity in sexual risk behavior (that is, some men may be more sexually active than others) and the possibility of using a targeted vaccination policy have been ignored in the current analysis; both of these issues will be addressed in subsequent analyses. However, previous results from modeling other diseases (6) suggest that in general, the inequalities shown in Eq. 4 are satisfied:

$$p_{het}{}^{u} > p_{hom}{}^{u} > p_{het}{}^{t} \quad (4)$$

where $p_{het}{}^{u}$ is the critical vaccination coverage level for eradication in a heterogeneous population with a uniformly applied vaccination program, $p_{hom}{}^{u}$ is the critical vaccination coverage level for eradication in a homogeneous population with a uniformly applied vaccination program, and $p_{het}{}^{t}$ is the critical vaccination coverage level for eradication in a heterogeneous population with a targeted vaccination program.

The inequalities shown in Eq. 4 reveal that the coverage levels for eradication are highest for a uniformly applied vaccination program in a heterogeneous population and lowest for a targeted vaccination program in a heterogeneous population. Therefore, if the mass vaccination programs are uniformly applied, then the calculated coverage levels in the current analysis (which assumes homogeneity) may be underestimates of the necessary coverage levels for epidemic eradication in San Francisco. However, if a vaccination program is targeted to the highest risk individuals, the calculated coverage levels in this current analysis may be overestimates of the necessary coverage levels for epidemic eradication in San Francisco. In addition, in the current analysis,

the possibility of revaccination has not been considered; if vaccines have low efficacy as a result of the waning of vaccine-induced immunity, then it may be very simple to increase vaccine efficacy by developing an appropriate revaccination schedule (*1*). Obviously, the development of an optimal vaccination campaign for HIV eradication may include the targeting of high-risk subgroups within the gay community and may include revaccination. The development of such optimal vaccination programs for HIV eradication therefore requires further theoretical exploration.

The results of our analysis suggest that extremely effective vaccines will have to be applied at high coverage levels to achieve HIV eradication (*10*). The available data indicate that it may be very difficult to achieve the necessary high participation rates unless highly efficacious vaccines are developed. The results demonstrate that risk behavior change and mass vaccination campaigns have to be considered together, and that it is extremely unlikely that vaccines will be able to eradicate HIV in San Francisco unless they are combined with considerable reductions in risk behaviors. If one of the consequences of a mass vaccination campaign is an increase in the level of risk behavior, the results indicate that it may become impossible to eradicate HIV. Although we wish to stress that if HIV eradication proves to be impossible, prophylactic vaccines (as we have shown elsewhere) could significantly reduce the HIV epidemic (*1*). However, the potential consequences of HIV mass vaccination campaigns need to be evaluated carefully, because (as we have shown in this analysis) such campaigns could result in a perverse outcome by increasing the severity of the epidemic. Therefore, the results illustrate that it is essential that efficacious prophylactic vaccines and efficacious behavioral intervention strategies be developed concurrently. A number of HIV prophylactic vaccines have already passed through phase I and phase II clinical trials. A recent decision has been made to delay phase III trials. This decision has the beneficial effect of allowing more time for the development of a quantitative theoretical framework for assessing the potential impact of prophylactic vaccines. We suggest that the developing theoretical framework should now be used in guiding the design of the phase III clinical trials, as well as in guiding the design of future mass vaccination campaigns.

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8. We obtained these estimates by using data from the SFYMHS and the equation $R_0 = \beta cD$ (*6*) (where β is the average transmission efficiency of HIV per sexual partnership, c is the average number of receptive anal sex partners experienced per unit time, and D is the average duration of infectiousness). To obtain the lower bound estimate we used risk behavior data only from the seronegative respondents, and to obtain the upper bound estimate we used risk behavior data from the entire cohort.
9. Any behavioral changes that affect either the level of condom use (which alters the value of β) or the rate of acquisition of receptive anal sex partners (which alters the value of c) will change the level of the risk behavior (βc). The initial value of βc was calculated from data from SFYMHS seronegative respondents (for the lower bound estimate) and from data from the entire SFYMHS cohort (for the upper bound estimate). In each case (either the upper bound estimate or the lower bound estimate) the initial value of βc was used as a standard and set to 1. We evaluated the risks of changing risk behavior by varying the relative level of the variable βc over the range 0 to 2. For each specific value of βc within this specified range of values we calculated the corresponding value of R_0 ($R_0 = \beta cD$). We then derived (using Eq. 2), for three specific efficacy levels (ϕ = 0.6, 0.8, and 1.0), the critical proportion that needed to be vaccinated in order to eradicate HIV.
10. In this analysis we have not presented any estimates of the number of years that it would take to eradicate HIV in San Francisco; eradication time estimates will be presented in a subsequent publication. The time to the eradication of HIV after a mass vaccination campaign has been initiated will be determined by several factors: the efficacy level of the vaccine, the attained vaccination coverage levels, the mechanism of action of the vaccine (that is, the relative contribution of take, degree, and duration), the incubation period of HIV, the surveillance criterion that is used to define eradication, the initial level of risk behavior, and the stability of risk behavior.
11. These data were collected by asking the respondents the following questions regarding their potential participation in a double-blind phase III vaccine efficacy trial: "Suppose you knew that the vaccine being tested was at least 80% effective—that is, at least 8 out of every 10 people who had received the vaccine would be protected against HIV infection. Would knowing this make you more or less likely to participate in a phase III vaccine trial or wouldn't that make any difference in your willingness to participate?" and "Suppose you knew that the vaccine being tested was somewhere between 40 and 60% effective—that is, between 4 and 6 out of every 10 people who had received the vaccine would be protected against HIV infection. Would knowing this make you more or less likely to participate in a phase III vaccine trial or wouldn't that make any difference in your willingness to participate?"

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Degradation of Gα by the N-End Rule Pathway

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The N-end rule relates the in vivo half-life of a protein to the identity of its amino-terminal residue. Overexpression of targeting components of the N-end rule pathway in *Saccharomyces cerevisiae* inhibited the growth of haploid but not diploid cells. This ploidy-dependent toxicity was shown to result from enhanced degradation of Gpa1, the α subunit (Gα) of a heterotrimeric guanine nucleotide–binding protein (G protein) that regulates cell differentiation in response to mating pheromones. Sst2, a protein whose absence renders cells hypersensitive to pheromone, was essential for degradation of Gα but not other N-end rule substrates, suggesting the involvement of an indirect, or trans-, targeting mechanism. Gα degradation by the N-end rule pathway adds another regulatory dimension to the multitude of signaling functions mediated by G proteins.

Many regulatory proteins are short-lived in vivo. The metabolic instability of a protein makes possible a rapid adjustment of its concentration or subunit composition through changes in the rates of its synthesis or degradation. The essential determinants of one degradation signal, named the N-degron, are a destabilizing NH_2-terminal residue and an internal lysine residue of a substrate protein (*1–3*). The lysine residue is the site of formation of a multiubiquitin chain, which comprises several covalently linked ubiquitin moieties (*2*, *4*). A set of N-degrons containing different destabilizing residues is manifested as the N-end rule, which relates the in vivo half-life of a protein to the identity of its NH_2-terminal residue (*1*, *2*). Ubiquitin is a 76-residue protein, the conjugation of which to other proteins plays a role in many cellular processes, primarily through routes that involve protein degradation (*4*). The only known physiological substrate of the N-end rule pathway has been the RNA polymerase

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