natural environment (*4, 22–24*) and that peptides for $CD4^+$ T cells can be inserted as guests in protein carriers (*25*).

Because Igs are self components devoid of the side effects sometimes associated with viral vaccines or viral proteins, exhibit a longer half-life than synthetic peptides, and can be internalized into APCs by means of FcγR, self Ig molecules that carry foreign T helper epitopes alone or in combination with a foreign B cell epitope could represent a new type of safe vaccine aimed at stimulating strong, specific immunity. This approach could also be extended to tumor-associated antigens and could represent a strategy to develop reagents able to stimulate antitumor immunity. The potential of chimeric self Ig-peptides as immunogens is enhanced by the fact that these constructs can be presented by dendritic cells, which are APCs known to be capable of capturing antigens in an immunogenic form in situ and of sensitizing naïve T cells in the absence of foreign adjuvants (*15, 16*). Because a T cell epitope can be generated from the CDR3 segment of a self Ig, it is possible that self Ig molecules with CDR3 segments having sequences recognized by T cells can contribute to autoimmune phenomena.

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14. Irradiated (2200 rads) spleen APCs (4×10^5) were incubated with graded quantities of either anti–I-E^d 14-4-4S or anti–I-A^d 25-9-17S. One hour later, Ig-HA (2 μg/ml) and HA(110–120) (5 μM) were added. After 2 hours, LD1-24 T cells (2×10^4) were added, and the mixture was incubated for 2 days. T cell activation was then measured as described in Table 1. In the presence of anti–I-E^d, 84% and 96% inhibition, respectively, of Ig-HA– and HA(110–120)–mediated T cell activation was obtained. No significant inhibition was obtained in the presence of anti–I-A^d.
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Evidence for a Clonal Origin of Methicillin Resistance in *Staphylococcus aureus*

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Soon after methicillin was introduced into clinical practice in the early 1960s, resistant strains of *Staphylococcus aureus* (MRSA) appeared, bearing a newly acquired resistance gene, *mecA*, that encodes a penicillin binding protein, PBP2a. MRSA have spread throughout the world, and an investigation of the clonality of 472 isolates by DNA hybridization was performed. All 472 isolates could be divided into six temporally ordered *mecA* hybridization patterns, and three of these were subdivided by the chromomosomal transposon Tn*554*. Each Tn*554* pattern occurred in association with one and only one *mecA* pattern, suggesting that *mecA* divergence preceded the acquisition of Tn*554* in all cases and therefore that *mecA* may have been acquired just once by *S. aureus*.

Staphylococcal resistance to methicillin (Mc^r), a frequent occurrence in both community and nosocomial settings (*1*), is a prime example of the development and spread of bacterial resistance after the introduction of an antibiotic into clinical practice. In Mc^r in *S. aureus*, a resistance determinant encodes a penicillin binding protein, PBP-2a (*2–4*), that binds β-lactams with much lower affinity than the native PBPs. Because PBP-2a is an addition to the standard set of PBPs, rather than a mutational modification of one of them, and was almost certainly acquired from some other organism after the introduction of methicillin (*5–7*), it presents an opportunity to study the evolution and spread of a single bacterial gene during a clearly defined time frame.

We report here the use of genomic DNA fingerprinting with variable gene (*8*) probes to construct an unambiguous temporally determinate (rooted) evolutionary tree encompassing over 450 Mc^r strains isolated during the past 30 years worldwide. The tree is based on molecular parsimony coupled with the temporal sequence of strain isolation and is consistent with the diversification of methicillin-resistant *S. aureus*

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(MRSA) from a single starting strain, as proposed by Lacey and Grinsted (*9*).

We found that the combination of two chromosomally located variable genetic elements showed enough variability to provide a rich source of strain-specific genotypic markers that, once established, are sufficiently stable to permit reliable inferences of coancestry within the 30-year time frame under consideration. In other words, they provided the appropriate resolving power for an analysis of this type.

We have used as probes the methicillin resistance gene (*mecA*) and Tn*554*, a transposon that is present in over 90% of MRSA isolates. Figure 1A shows six different blot-hybridization patterns obtained with the *mecA* probe. Among our 472 independent isolates, we have encountered only these six Cla I restriction fragment length polymorphisms (RFLPs). The six patterns can be arranged in a simple chronological tree (Fig. 1B), in which adjacent branches differ by only one single genetic event (that is, mutation, deletion, or insertion). Thus, pattern VI, as shown by the earliest known isolate (from 1961) and seen only once in our set, is placed at the top of the tree; pattern II, predominant in the early 1960s, is below; and the other patterns, first seen in the 1980s, are assumed to have arisen from pattern II.

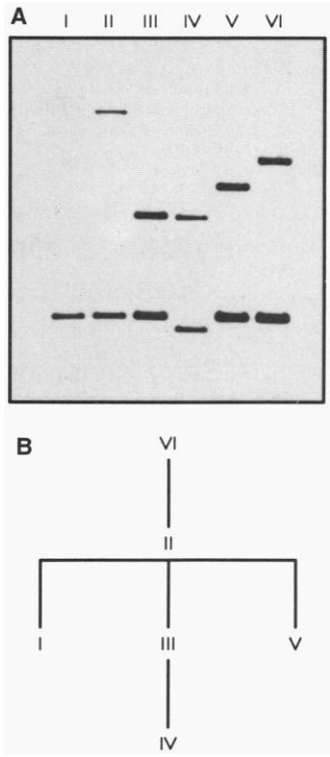

Fig. 1. (**A**) Polymorphism involving *mecA*. Cla I digests of whole cell DNA were electrophoresed in 1% agarose, transferred to Nytran (S&S), and probed with a *mecA*-specific radiolabeled DNA probe (*20, 25*). A total of 472 MRSA isolates were fingerprinted, and the year of isolation and source of the strains are summarized below. For *mecA* type I, 245 strains were isolated from the mid-1980s to the present from New York City, New Jersey, Pennsylvania, Wisconsin, Dublin (Ireland), Montreal (Quebec), Edmonton (Alberta), and Manitoba. For *mecA* type II, 166 strains were isolated from 1961 to the present from New York City, New Jersey, Dublin (Ireland), Denmark, England, Uganda, Nairobi (Kenya), Cairo (Egypt), Geneva (Switzerland), London (Ontario), Toronto (Ontario), Halifax (Nova Scotia), Winnipeg (Manitoba), and Saskatoon (Saskatchewan). For *mecA* type III, 16 strains were isolated from the late 1980s to the present from New York City, New Jersey, Dublin (Ireland), Pennsylvania, Montreal (Quebec), and Manitoba. For *mecA* type IV, 41 strains were isolated from 1982 to the present from New York City, Australia, and London (England). For *mecA* type V, two strains were both isolated from the late 1980s, one from Manitoba and the other from Toronto, Ontario. The lone isolate that defines *mecA* type VI is a 1961 strain (the earliest MRSA isolate in our collection) from Cairo, Egypt. (**B**) Possible evolutionary tree for the *mecA* polymorphism in MRSA. The overall tree is based on apparent chronology inferred from the time of first isolation of each type. Branches connect types that could have arisen from one another by a single genetic event.

Given the small number of *mecA* hybridization patterns, a second probe was needed to provide a higher degree of resolution. We chose Tn*554* because it was by far the most common among MRSA isolates of several transposon probes examined (*14, 15*) and has never been found naturally on a plasmid. Among 3398 MRSA from more than 40 hospitals, 3085 (approximately 91%) contained Tn*554* on the basis of spectinomycin resistance, a marker that is uniquely associated with Tn*554* in *S. aureus* (*16*). Tn*554*, a transposon with very limited site preference (*16–18*), occurs at several different sites in *S. aureus* with differing frequencies and is often present in two or more copies (*19*). Thus far, we have identified 29 different Tn*554* blot-hybridization patterns among MRSA isolates, which we consider to be a sufficient amount for definitive evolutionary and epidemiological analysis. Examples of these patterns are shown in Fig. 2. Unlike the *mecA* patterns, the available Tn*554* patterns, even those within a single *mecA* hybridization type, cannot be used to construct an unambiguous tree based on single genetic steps. This may indicate the lack of isolates representing intermediate steps, multiple independent acquisitions of the transposon, or complex internal rearrangements from single starting points.

The interpretation of data such as these requires an assessment of the stability of the

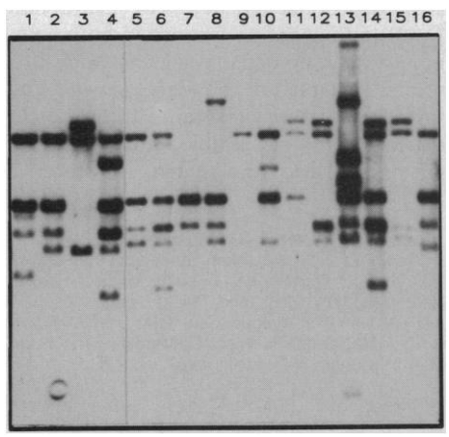

Fig. 2. Polymorphism involving Tn*554*. The same membranes probed with *mecA* were either stripped by boiling or stored until the radioactive signal had decayed. A radiolabeled Tn*554*-specific DNA probe (*14, 15, 20*) identified 29 different Cla I hybridization patterns, and each has been assigned a letter code. Fifteen of these patterns are shown. For each pattern shown, the *mecA*-Tn*554* type, the number of representative isolates, and the source are listed. Lane 1, I:U, 1 isolate, New York City; lanes 2 and 5, I:A, 214 isolates, New York City, New Jersey, Pennsylvania, Wisconsin, Dublin (Ireland), Montreal (Quebec), and Manitoba; lane 3, II:V, 1 isolate, Dublin (Ireland); lane 4, III:W, 1 isolate, Dublin (Ireland); lane 6, III:B, 9 isolates, New York City, New Jersey, Montreal (Quebec), and Manitoba; lane 7, I:C, 3 isolates, Alberta; lane 8, II:D, 29 isolates, New York City, Toronto, and London (Ontario); lane 9, II:E, 11 isolates, New York City, Dublin (Ireland), Saskatchawan, Manitoba, England, and Denmark; lane 10, II:F, 2 isolates, New York City and London (Ontario); lane 11, I:G, 5 isolates, New York City; lane 12, II:J, 43 isolates, New York City, New Jersey, and Toronto (Ontario); lane 13, II:L, 3 isolates, Toronto (Ontario); lane 14, IV:M, 41 isolates, New York City, Australia, and England; lane 15, V:O, 2 isolates, Toronto (Ontario) and Manitoba; lane 16, I:P, 10 isolates, New York City.

markers used. Accordingly, we tested the stability of several of the patterns shown in Fig. 2 by repetitive subculture and blot hybridization. The results (shown in Fig. 3) demonstrate that these patterns are absolutely stable over a 6-week period, which represents approximately 1000 generations of bacterial growth.

Examination of the six *mecA* and 29 Tn*554* blot-hybridization pattern types revealed the general rule that each Tn*554* pattern occurs in association with one, and only one, *mecA* pattern, suggesting that primary differentiation of *mecA* patterns is followed by independent evolution of the Tn*554* patterns within each *mecA* family. This rule is illustrated in Fig. 4, in which each set of Tn*554* patterns has been placed with the *mecA* pattern with which it is uniquely associated. The chronology implied in this scheme is supported by the observation that the vast majority of MRSA isolates lacking Tn*554* belong to MRSA type II, the early type from which, we suggest, all the others have descended. Furthermore, Mc^s strains containing Tn*554* show Tn*554* hybridization patterns different from any seen with MRSA (*15*). Tn*554* contains a single Cla I restriction site, so that single transposon insertions yield two hybridizing bands (Fig. 2, lane 9). Some of the patterns with multiple bands clearly represent multiple insertion; deletions or other DNA rearrangements are probably also present (*19*).

Overall, these results suggest that horizontal transfer of *mecA* after its initial establishment in *S. aureus* is extremely rare. This tree therefore constitutes a clearly defined evolutionary framework within which other strains and other markers can be evaluated as well as a means of establishing epidemiological relationships among strains unequivocally. It appears, therefore, that genomic hybridization with variable gene probes has the appropriate resolving power for the analysis of clinical MRSA isolates.

We have tracked a number of MRSA outbreaks and found without exception that common-source isolates have the same *mecA*-Tn*554* pattern, whereas noncommon source isolates from the same locale have different patterns (*14, 15, 20*). Similar studies for *Mycobacterium tuberculosis* used an IS element (*21*). We hope that systems of this type will find wider application in evolutionary-epidemiologic analysis; a limitation, however, is that probe sequences occurring on plasmids or prophages should be avoided because these will introduce an unacceptable level of variation.

We suggest finally that a genotyping system based on defined chromosomally located variable genetic elements provides significant advantages over systems using purely phenotypic variables, such as exoprotein patterns, phage susceptibility patterns, and most serotypes, because the genetic basis of the phenotypic variability is usually unknown and the observed phenotypic variations can often be caused by more than one type of genetic event (*8, 22*). A particular example is that of phage typing for *S. aureus*, a system that lacks any systematic biological basis and is plagued by nontypable isolates and by unpredictable and uninterpretable variability among typable ones. This system has long outlived its usefulness and is clearly in need of replacement by a rigorous genotyping system such as that described here.

Note added in proof: Recent observations by Musser and Kapur (*23*) and by Archer and co-workers (*24*) suggest that *mecA* may have entered *S. aureus* on one

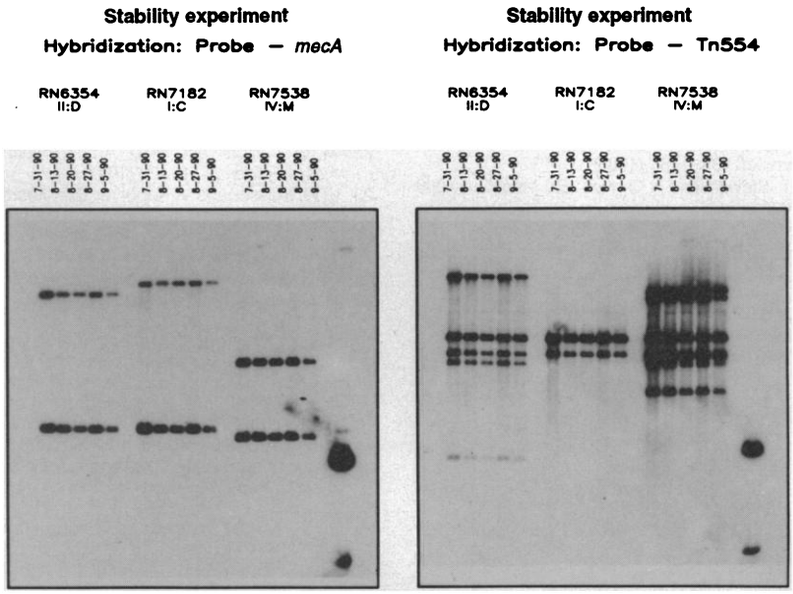

Fig. 3. Stability of *mecA*-Tn*554* blot hybridization patterns. Three isolates coded as II:D, I:C, and IV:M were serially passed on both nonselective GL agar (*26*) for 6 weeks. A single colony was streaked daily from Monday through Friday, and DNA was isolated from each Friday culture. The dates over each lane identify samples prepared from 5 of the 6 weeks.

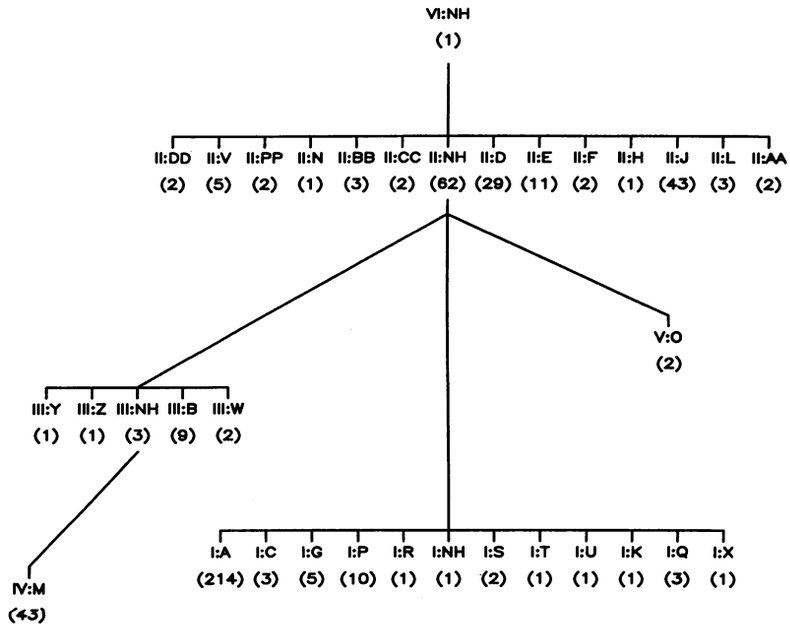

Fig. 4. Proposed *mecA*-Tn*554* evolutionary tree. The letter-coded Tn*554* patterns have been superimposed on the *mecA* tree shown in Fig. 1 so that each set of Tn*554* branches represents the letter-coded Tn*554* patterns uniquely associated with that *mecA* pattern. Values in parentheses represent the total number of isolates with that fingerprint. NH, no homology with Tn*554* probe.

or possibly two recent occasions in addition to the original one proposed. This might mean that one or two of the *mecA* polymorphisms shown in Fig. 1A could have arisen in the putative donor species before horizontal transfer to *S. aureus*. Any such de novo progenitor could then have given rise independently to one of the *mecA*-based groups shown in Fig. 4.

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Regulation of Heat Shock Factor Trimer Formation: Role of a Conserved Leucine Zipper

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The human and *Drosophila* heat shock transcription factors (HSFs) are multi-zipper proteins with high-affinity binding to DNA that is regulated by heat shock–induced trimerization. Formation of HSF trimers is dependent on hydrophobic heptad repeats located in the amino-terminal region of the protein. Two subregions at the carboxyl-terminal end of human HSF1 were identified that maintain the monomeric form of the protein under normal conditions. One of these contains a leucine zipper motif that is conserved between vertebrate and insect HSFs. These results suggest that the carboxyl-terminal zipper may suppress formation of trimers by the amino-terminal HSF zipper elements by means of intramolecular coiled-coil interactions that are sensitive to heat shock.

Organisms respond to mild heat stress and to a variety of chemical inducers by rapidly increasing the transcription and translation of heat shock protein genes (*1*). The synthesis of heat shock proteins leads to an increased concentration of molecular chaperones, which are thought to maintain the native state and folding of cellular proteins under conditions of physiological stress (*2*). In eukaryotes, a preexisting transcriptional activator, HSF (*3*), mediates activation of heat shock genes by binding to conserved, upstream response elements [heat shock elements (HSEs)] (*4*). HSF binds to the HSE with high affinity as a trimer of identical subunits (*5*, *6*).

The synthesis of HSF protein is not regulated by heat shock, but the high-affinity binding of HSF to DNA is dependent on heat shock. The heat shock–inducible binding of HSF to the HSE in *Drosophila*, vertebrates, and plants but not in yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis* (*3*, *7–9*) requires a transition of the HSF protein (*10*). Gel-filtration chromatography, sedimentation analysis, and chemical cross-linking of the two forms of *Drosophila* HSF indicate that this transition is a conversion from monomer to trimer (*11*); studies of the human HSF1 protein have found a similar change (*12*). In *S. cerevisiae*, the absence of control over HSF binding to DNA is reflected by the constitutive formation of HSF trimers, which can occupy chromosomal HSEs in vivo under normal as well as under heat shock conditions (*13*). Control of *S. cerevisiae* HSF activity is exercised at the level of transcriptional activation, which is correlated with increased phosphorylation at a number of serine and threonine residues (*14*). Increased phosphorylation after heat stress has also been observed for human HSF (*12*, *15*), but the mechanism by which phosphorylation may activate HSF is unknown (*9*, *14*).

Our efforts to understand the stress signal transduction pathway have focused on the mechanism by which heat shock leads to the aggregation and high-affinity binding of HSF protein. The *Drosophila* and human HSF proteins synthesized in *Escherichia coli* form trimers, hexamers, and higher oligomers at nonshock temperatures, suggesting that the HSF polypeptide has an intrinsic ability to form aggregates (*12*, *16*). The formation of trimers of HSF is dependent on several arrays of evolutionarily conserved, hydrophobic heptad repeats (zipper motifs) located next to the DNA-binding domain at the NH_2-terminal end of the protein (*5*, *16*). Thus, the stability of the HSF monomer under normal conditions could be dependent on a mechanism that suppresses the aggregation of the NH_2-terminal zipper elements.

A comparison of the predicted sequences of HSF proteins cloned from *S. cerevisiae*, *Drosophila*, and a human source provides insight into the mechanism for trimer suppression. Whereas HSF proteins of all three species contain conserved sequences in the NH_2-terminal DNA-binding domain and the adjacent zipper motifs, only *Drosophila* and human HSF proteins have an additional hydrophobic heptad repeat in the COOH-terminal region (*16*, *17–19*). Because this fourth zipper is absent from the constitutively trimeric yeast HSF, we suggested that it could be involved in the suppression of the aggregation of the metazoan HSFs under normal conditions (*17*).

To test this hypothesis, we changed two hydrophobic residues in the fourth zipper

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