Other Applications of Stochastic Cooling; Future Developments

At present, stochastic cooling is used at CERN in the AA ring and in the Low Energy Antiproton Ring (LEAR) where the $\bar{p}$'s may be stored after deceleration in the PS. Before the Intersecting Storage Rings (ISR) were closed down last year, they also used the antiprotons and contained cooling equipment.

In the SPS where the high-energy collisions take place, cooling would be attractive because it would improve the beam lifetime and might decrease its cross section. However, a difficulty is formed by the fact that the beam is bunched in this machine; the bunches are narrow (3 or 4 nanoseconds). In fact, owing to the bunching, each Schottky band is split into narrow, dense satellite bands, and the signals from different bands are related (*20*). Nevertheless, a scheme is being considered that might improve the lifetime to a certain extent (*21*).

In the United States, a $\bar{p}$ accumulator complex similar to the one at CERN and also using stochastic cooling is being constructed (*22*). This machine is expected to have a stacking rate an order of magnitude higher than the one at CERN, because it uses a higher primary energy to produce the antiprotons and higher frequencies to cool them.

In the meantime, we are building a second ring at CERN, surrounding the present accumulator (Fig. 13) with a similar performance. It will have stronger focusing, thereby increasing both transverse acceptances by at least a factor of 2 and increasing the longitudinal one by a factor of 4. The increased focusing strengths will diminish the mixing; consequently, higher frequencies (up to 4 GHz) will be used for cooling. The present AA will be used to contain the stack and its cooling systems will also be upgraded.

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23. The development of the stochastic cooling theory owes much to H. G. Hereward, D. Möhl, F. Sacherer, and L. Thorndahl. The latter also made important contributions to the construction of most cooling systems at CERN, and it is doubtful if the accumulator would have been feasible without his invention of the filter method. It is also a pleasure to acknowledge the invaluable contributions of G. Carron (hardware), L. Faltin (slot pick-ups), and C. Taylor (1- to 2-GHz systems). During the construction of the CERN Antiproton Accumulator, R. Billinge was joint project leader with me. It is mostly because of his contributions to the design and his able management that the machine was ready in a record time (2 years) and worked so well.

RESEARCH ARTICLE

Control of Directionality in Lambda Site Specific Recombination

Wade Bushman, John F. Thompson, Lina Vargas, Arthur Landy

Site-specific recombination of DNA is an integral aspect of a wide range of biological processes such as viral development, transposition, antigen variation, immunoglobulin diversification, and oncogenesis. An important and interesting aspect of these processes is the mechanisms by which physiological factors are incorporated into the regulation of their timing and direction. The lysogenic pathway of bacteriophage lambda affords a tightly regulated recombination system that is particularly amenable to analysis of these questions. We show here that viral integration and excision make asymmetric use of protein binding sites and display marked differences in their dependence on the concentration of the relevant proteins. The two recombinations occur along discrete pathways that are different in several ways, some of which generate a mechanism for growth phase-dependent control of recombination.

During establishment of lysogeny, integrative recombination between a 250–base pair (bp) site on the phage chromosome (*att*P) and a 25-bp site on the bacterial chromosome (*att*B) yields an integrated prophage bounded by two prophage *att* sites, *att*L and *att*R (Fig. 1) (*1, 2*). The apparent reversal of this reaction leads to excision of the prophage by recombination between *att*L and *att*R to regenerate *att*P and *att*B. The exchange of DNA strands is accomplished by a 7-bp staggered cut in each recombining partner within a region that is homologous in all four *att* sites (*3*). DNA homology within, but not beyond, the 7-bp overlap region is crucial for recombination, which is thought to proceed through a cruciform (or "Holliday") intermediate via a set of closely coupled strand exchanges (*4–7*).

Integrative recombination is executed by the phage-encoded protein integrase (Int) along with the bacterially encoded protein IHF (integration host factor). The integrase, Int, can bind sites flanking the overlap region in all four *att* sites (*8*). When bound at these "core-type" sites, Int effects strand exchange by nicking and ligating the DNA, one strand at a time (*7*), in the absence of any added energy source (*9*). It also binds several sites in the arm regions of *att*P. Because the consensus sequence for these arm-type sites differs from that of the core-type sites, Int is postulated to have two distinct DNA binding domains (*10*). Al-

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though IHF, which binds to three sites in *att*P (*11*), is normally required for recombination, its role as an "accessory protein" is highlighted by the isolation of Int mutants capable of carrying out recombination in the absence of IHF (*12, 13*). In addition to its role in recombination, IHF is involved in the regulation of several bacterial processes and mutants display pleiotropic deficiencies (*14–16*). The binding of these proteins to *att*P results in the formation of a discrete protein-DNA complex ("intasome") that can be visualized in the electron microscope (*17*); and, on the basis of nuclease protection experiments and topological analyses of recombination, this complex appears to have DNA wrapped around or along the protein core with the same handedness as that of a nucleosome (*18,19*).

Excisive recombination is distinguished by requiring the phage-encoded protein excisionase (Xis) in addition to Int and IHF. Excisionase has been shown to bind cooperatively to adjacent sites on the P arm of *att*P and *att*R (*20*), and is required together with Int for the formation of an intasome on *att*R (*21*). In addition to promoting excisive recombination, Xis inhibits integrative recombination (*22, 23*). The directionality is not determined by the bacterial DNA sequences, but rather it is governed by the relative locations of the phage arms on the same *att* site (*att*P) in integrative recombination or on different *att* sites (*att*L and *att*R) in excisive recombination (*24*).

The pivotal role of Xis has understandably dominated most models for directionality in lambda site-specific recombination. Our data indicate that Xis is not the sole controlling factor; instead, there is a complex interplay among all three recombination proteins. Furthermore, the concentration of intracellular IHF varies in a growth phase–dependent manner and may play as large a role as Xis in determining the direction of recombination.

Integrative and excisive recombination require different extents of DNA. The bacterial DNA beyond the core-type Int binding sites has been shown to be unimportant for the integration and excision reactions (*24, 25*). In order to compare the extent of phage DNA required for recombination, sets of altered phage and prophage *att* sites have been constructed by progressively deleting the outermost phage DNA and replacing it with heterologous DNA. This results in three sets of altered *att* sites (*att*P, *att*L, and *att*R), which are lacking one or more protein

Abstract. *The simple relation between the substrates and products of site-specific recombination raises questions about the control of directionality often observed in this class of DNA transactions. For bacteriophage lambda, viral integration and excision proceed by discrete pathways, and DNA substrates with the intrinsic property of recombining in only one direction can be constructed. These pathways display an asymmetric reliance on a complex array of protein binding sites, and they respond differently to changes in the concentrations of the relevant proteins. The* Escherichia coli *protein integration host factor (IHF) differentially affects integrative and excisive recombination, thereby influencing directionality. A four- to eightfold increase in intracellular IHF coincides with the transition from exponential to stationary phase; this provides a mechanism for growth phase-dependent regulation of recombination that makes the cellular physiology an intrinsic part of the recombination reaction.*

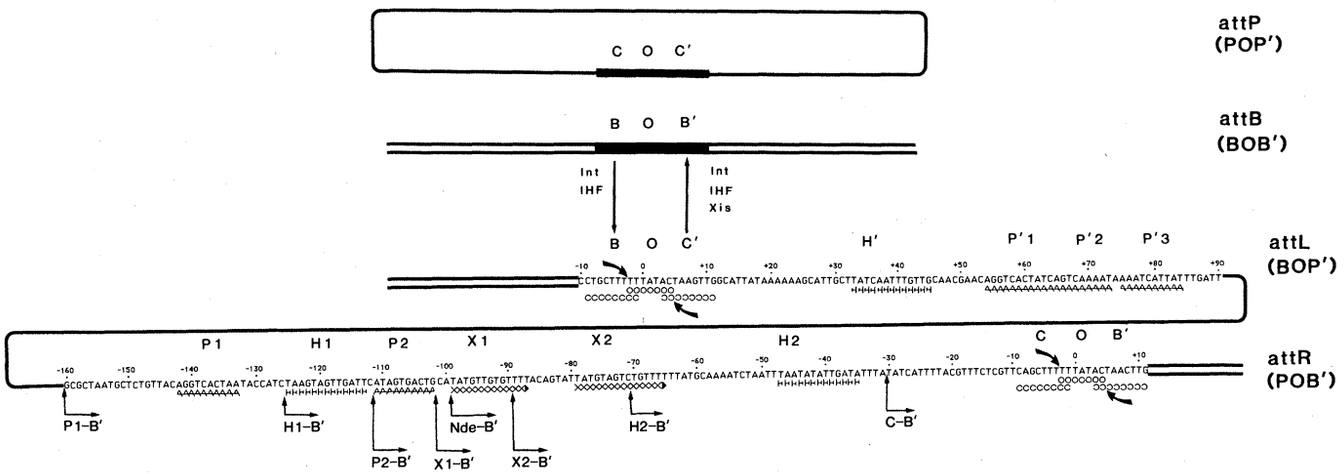

Fig. 1. The DNA substrates, proteins, and protein binding sites in lambda site specific recombination: Circular phage DNA (*att*P, ——), and linear bacterial DNA (*att*B, ═) undergo integrative recombination to yield *att*L and *att*R. For *att*P and *att*B, the core-type Int sites and overlap regions (COC' and BOB', respectively) are indicated. For *att*L and *att*R, which can undergo excisive recombination to yield *att*P and *att*B, the top strand DNA sequence is shown (*49*). The protein binding sites for IHF (H), Xis (X), arm-type Int (P), and core-type Int (C or B) are numbered from left to right and marked with prime signs if present to the right of the overlap region. Although the regions underscored with symbols include all of the bases apparently important for specific protein recognition, some of the interior bases are not specified in the consensus sequences (*8, 10, 11, 20, 50*). The relative orientation of sites for a given protein is indicated by the symbols (IHF: H or H, arm-type Int: A or A, core-type Int: c or ɔ, and Xis: ×▶). The sites of strand exchange in the top strand (◥) and omitted bottom strand (◥) demark the 7-bp overlap region (O). The resected *att*R sites (lower portion) are named for their outermost complete protein binding site as described in the text (Nde-B' refers to an *att*R cut with Nde I prior to use in order to remove all DNA to the left of −99). The last base for which each resected *att*R matches the unresected *att* DNA is shown (▲). Most resected *att*R plasmids were constructed from *att*P plasmids by in vitro recombination. The construction of P1-P'3, P2-P'3, X2-P'3, H2-P'3, and C-P'3 has been described (*18*). H1-B' was made by (i) digestion of P1-B' with Dde I, (ii) filling in the staggered ends with the Klenow fragment of DNA polymerase, (iii) gel purification of the appropriate sized fragment on low-melting agarose, and then digestion with Bam HI. This was ligated to the large Eco RV-Bam HI fragment of pBR327. X1-B' was made by cutting P1-B' and pBR322 with Nde I and Bam HI. The appropriate fragments were then ligated. B-H' (end point at +46), B-P'1 (+64), and B-P'3 (+100) were made by recombination in vitro with previously described resected *att*P plasmids (*18*). Constructions that are missing different extents of the same protein binding site are not shown separately because identical results were obtained within such sets.

binding sites on the phage arms. The different *att* sites used in our study are summarized in Fig. 1 and are described by a shorthand notation which lists the outermost intact protein binding sites on both sides of the overlap region. For example, the unresected *att*R is written P1-B′, while an *att*R lacking the P1 site is designated H1-B′.

The competence of the resected *att* sites for integration (*att*P) and excision (*att*L and *att*R) was tested in an in vitro recombination reaction with purified components (Fig. 2) (*26*). Removal of either the P1 site from the P arm or the P′2 and P′3 sites from the P′ arm renders *att*P incompetent for integrative recombination. The different *att*P sites were tested with a wide range of Int and IHF concentrations and in different topological states. Consistent with previous results, integration involving the unresected *att*P is optimal with *att*P supercoiled and *att*B linear, but all shortened *att*P sites were unable to recombine to a significant extent with *att*B under any conditions. Together with earlier results obtained with a less complete set of resected *att*P sites in a crude recombination system (*18, 27*), these observations show that the span of phage *att* DNA required for *att*P function includes even the outermost protein binding sites.

When similar tests are carried out with the resected *att*R sites, a much different result is obtained. If P1 or H1 is removed, no deficiency in Xis-dependent recombination with *att*L is seen under any conditions. Even when P2 or part of X1 is deleted, excision can still occur, albeit in a more restricted set of conditions. However, when X2 is also removed, there is no detectable recombination with *att*L either in the presence or absence of Xis. Removal of the P1, H1, P2, and X1 sites does not abolish the requirements for any of the three proteins in recombination. To rule out the possibility that heterologous DNA adjoining the resected *att*R end points was fortuitously contributing to recombination function, we used four entirely different pBR322 sequences to form the junction with *att* DNA and observed no differences for these constructions. In fact, the DNA can be deleted entirely from beyond X1 by digestion with the restriction endonuclease Nde I. This results in a linear molecule that can recombine efficiently even though there is no DNA where P1, H1, and P2 normally are present.

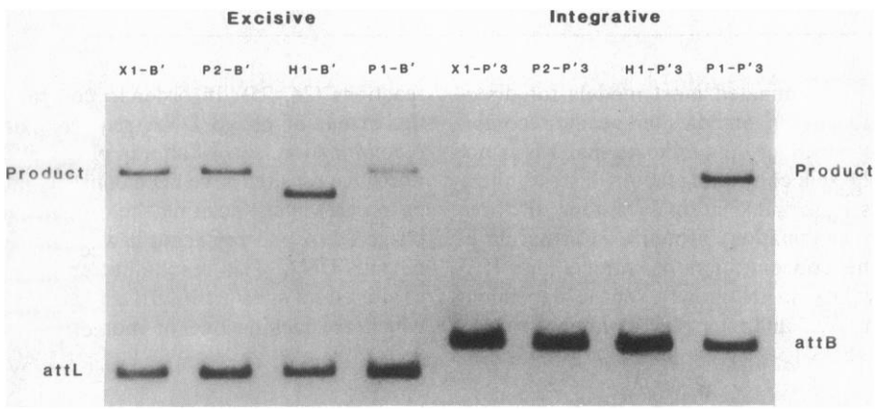

Fig. 2. Excisive and integrative recombination with resected *att* sites. Excisive recombinations with supercoiled *att*R and radioactive linear *att*L; and integrative recombination, with supercoiled *att*P and radioactive linear *att*B, were carried out in vitro (*24*). The identity of the *att*R and *att*P substrates used in the reactions is indicated at the top of each lane. *att*L and *att*B were linearized with Eco RI and labeled with [α-^{32}P]dATP and the Klenow fragment of DNA polymerase. Labeled DNA's were 1.25×10^{-11} *M* in the 20-μl sample. Purified proteins were added to the DNA-buffer mix in the following order and concentrations (for excision and integration, respectively): IHF (2.5 n*M* and 10 n*M*), Int (62 n*M* and 125 n*M*), and Xis (150 n*M* and 0 n*M*). After 4 hours at 25°C, 10 μl of "loading" solution [1 percent SDS, 10 percent Ficoll, salmon sperm DNA (25 μg/ml)] were added to each sample and placed onto a 1.2 percent agarose gel. After electrophoresis for 16 hours at 40 V, the gels were dried and autoradiographed. The product for the reaction of H1-B′ with *att*L is smaller than for reactions involving the other *att*R DNA's because it is on the smaller pBR327 backbone rather than pBR322.

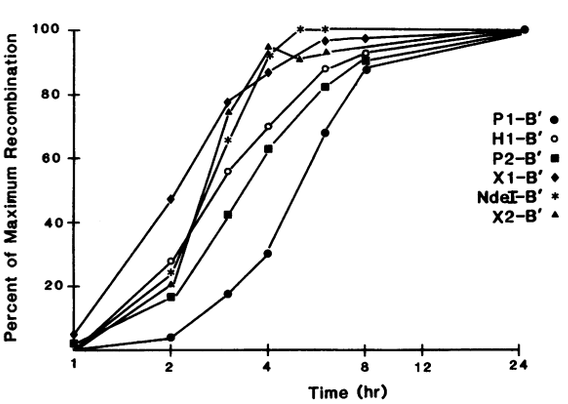

Fig. 3. Kinetics of excisive recombination: Labeled *att*R substrates were recombined with supercoiled *att*L as described in Fig. 2, except that IHF, Int, and Xis were present at 1.25 n*M*, 25 n*M*, and 150 n*M*, respectively, and reactions were done at 20°C so that kinetics could be more accurately studied. Similar results are obtained at 25°C. Autoradiograms were analyzed with a Hoefer GS300 scanning densitometer attached to a Hewlett-Packard 3390A integrator. Percentages of recombination for each time point were normalized to the percentage obtained at 24 hours for each combination of *att* sites. The values for the efficiency at 24 hours were 39 percent (P1-B′), 41 percent (H1-B′), 43 percent (P2-B′), 48 percent (Xl-B′), 25 percent (Nde-B′), and 12 percent (X2-B′).

The competent resected *att*R sites share many important characteristics with the unresected *att*R. Recombination is dependent on Xis (as well as Int and IHF) and can occur with either *att*L or *att*R supercoiled or with both linear (*28*). Between 18° and 30°C, all excisions are fully efficient. At 33°C, each reaction is reduced in efficiency and, at 37°C, no recombination is observed (*28*).

The rate of recombination for each of the *att*R sites with supercoiled *att*L was examined. When the normalized rates of excision are compared, the unresected *att*R is the slowest to reach its maximum level of recombination (Fig. 3). Even when the recombination data are plotted as an absolute rate, H1-B′, P2-B′, X1-B′, and Nde-B′ all proceed faster than P1-B′.

The resected *att*L sites were also tested for their competence in excisive recombination. While linear or supercoiled B-P′3 can efficiently recombine with linear or supercoiled *att*R, both B-P′1 and B-H′ are unable to recombine with either the full-length or shortened *att*R's under any set of topological conditions. This inability to recombine is observed even when the concentrations of the three proteins are varied over a wide range (*28*). These observations do not allow us to resolve any differences between integration and excision arising from the P′ arm; however, a mutant with a single base change in P′3 has been characterized that restricts integration much more than excision (*29*). This same base change has also been introduced into

each of the other arm-type Int binding sites by Bauer and Gardner whose results also indicate that the requirements on the P′ arm are different for integration and excision (*30*).

Dependence of excisive recombination on protein concentrations. In order to define what influence, if any, the nonessential P arm binding sites exert on the excision reaction, the behavior of the intact and resected *att* sites was scrutinized under varying Int, Xis, and IHF concentrations. The recombination reactions that we describe were carried out with the concentration of *att*R below the apparent dissociation constant (K_D) for each of the three proteins such that the true concentration dependence is observed (rather than the stoichiometric requirement). While it is possible that a low affinity of *att*L for the proteins could mask any differences among the *att*R sites, our nuclease protection data show that, when Int and IHF are added together, they bind to *att*L at lower concentrations than *att*R (*28*). Furthermore, *att*L does not bind Xis (*20*), and therefore any differences found can be attributed to differences among the *att*R sites.

When the concentration of Int is varied, we find that the level of Int needed to initiate recombination for P2-B′, X1-B′, and X2-B′ is less than that needed for the unresected *att*R (Fig. 4). The resected *att*R sites recombine to a greater extent than the unresected *att*R under these conditions (high Xis concentration and supercoiled *att*L) (*31*).

The resected *att*R sites have very different patterns of recombination efficiency in response to changes in the Xis concentration (Fig. 5). For example, P1-B′ and P2-B′ are virtually identical in their concentration dependence for Xis, X1-B′ needs approximately four times as much Xis, Nde-B′ needs eight times as much, and X2-B′ needs 16 times as much. These differences reflect the disruption of cooperative interactions that enhance Xis binding to *att*R (*24*). There is positive cooperativity in the binding of Xis to X1 and X2; in addition, Xis binding to *att*R is stimulated by Int bound at P2. Therefore, the apparently dispensable P2 and X1 sites, at the very least, facilitate *att*R function by enhancing Xis binding at limiting concentrations.

Additional observations suggest that the X1 and P2 sites may play a more important role than simply enhancing Xis binding. When nuclease protection was used to examine Xis binding to X2-B′, it was observed that weak protection of the X2 site at high Xis concentrations is accompanied by a weaker protection of the adjacent heterologous DNA (*24*). This suggests that X2-B′ may be competent for excision at high Xis concentrations only because Xis binding to X2 induces Xis binding to the adjacent DNA, thus mimicking the binding of Xis to a normal *att*R. Similarly, in X1-B′, Xis induced Int to protect adjacent DNA even though the P2 site is absent (*28*), again indicating that protein-protein interactions are an important component of binding specificity in the recombination complex. Thus, in *att*R constructions lacking a proper P2 site, Int could occupy an analogous place in the complex, held in place by direct interactions with Xis and possibly by interactions with a core-type Int site.

IHF inhibition of excisive recombination. When the IHF concentration is varied, the pattern of excisive recombination at low concentrations is the same for all *att*R sites tested, but there is a significant difference at higher IHF con-

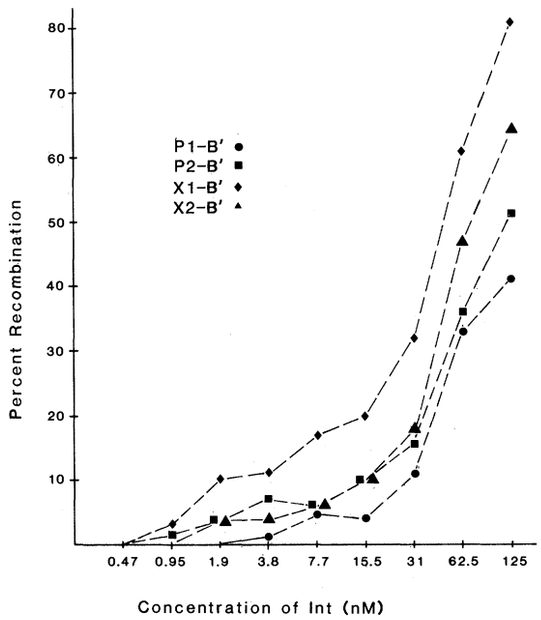

Fig. 4. Int dependence of excision. The concentration of Int was varied in twofold increments under the recombination conditions described in Fig. 3, except that IHF and Xis were present at 5 n*M* and 150 n*M*, respectively. The abscissa is a logarithmic scale and the ordinate is the absolute percentage of each *att*R recombined (not the fraction of the final recombination as in Fig. 3).

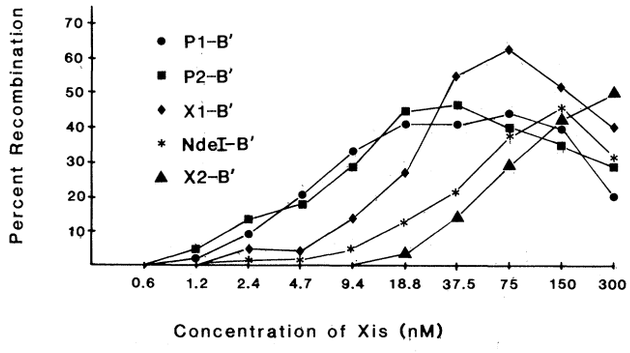

Fig. 5. Xis dependence of excision. The concentration of Xis was varied in twofold increments (and plotted on a logarithmic scale) under the recombination conditions described in Fig. 3. IHF and Int were 5 n*M* and 60 n*M*, respectively.

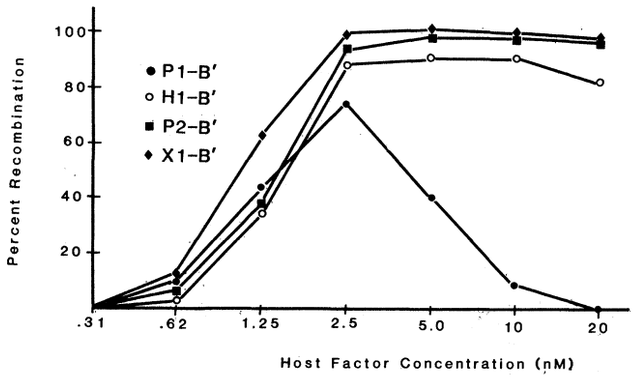

Fig. 6. IHF dependence of excision. The concentration of IHF was varied in twofold increments (and plotted on a logarithmic scale) as described in Fig. 3. Int and Xis were 12 n*M* and 150 n*M*, respectively.

centrations (Fig. 6). The recombination efficiency of P1-B′ drops dramatically when the IHF concentration is above 2.5 n*M*; in contrast, the shortened *att*R sites continue to recombine efficiently before succumbing at very high IHF concentrations to the same inhibition that is also seen with integrative recombination.

The requirement for IHF in excisive recombination coupled with its specific inhibitory effect at higher concentrations suggests that one or more IHF binding sites are required for excision while one or more sites are inhibitory. Since H1 is the weakest of the three sites by nuclease protection (*28*) and altering the sequence around the H1 site removes the inhibition, binding of IHF to H1 likely mediates IHF inhibition of excision. Integration requires more IHF than does excision for optimal reaction (*16, 32, 33*) and is not inhibited specifically, as is excision. This suggests that the H1 site affects the two reactions differently. Indeed, IHF binding at H1 may play as large a role in controlling the direction of recombination as does Xis. The exact mechanism by which IHF can carry out these functions must await a thorough analysis of mutant *att* DNA's that are defective in the individual protein binding sites. These studies should also illuminate the protein-protein interactions that are suggested by the lack of IHF inhibition in H1-B′ (Fig. 6).

Table 1. Properties of resected *att* DNA's. The resected end point, outermost intact phage-arm protein binding site, and plasmid name are given for all DNA's used. The *att*P plasmids carry a complete P or P′ arm in combination with the resected arm while the *att*L and *att*R plasmids carry a complete B or B′ arm with the resected P′ and P arm, respectively. Also shown is the ability of the resected *att* DNA to recombine with the appropriate partner in integrative and excisive recombination, the relative rate of excision, the capacity of IHF to inhibit excision, and the relative concentration of Xis required for excision. NA and ND stand for not applicable and not determined.

Resected end point	*att*P	*att*R or *att*L	Function		Excision (relative rate)	IHF inhibition	Relative Xis required
			Integration	Excision			
−251 } P1	WR1	PH202	+	+	1	Yes	1
−160 } P1	PH53	WB53-1					
−125 H1	JT17	JT16	−	+	>1	No	1
−111 P2	PH2-38	WB55-38	−	+	>1	No	1
−109	PH55	WB55-100					
−108 } X1	PH2-21	WB55-21	−	+	>1	No	4
−102		WB55-102					
−99 Nde			−	+	>1	No	8
−89 X2	PH56	PH56-1	−	+	>1	ND	16
−70 } H2	PH57	WB57-1	−	−	0	NA	NA
−50 } H2	PH58	WB58-1					
−30 C	PH59	WB59-1	−	−	0	NA	NA
+242 } P′3	WR1	PH201	+	+	1	Yes	1
+100 } P′3	PH304	WB104					
+64 P′1	PH308	WB108	−	−	0	NA	NA
+46 H′	PH310	WB110	−	−	0	NA	NA

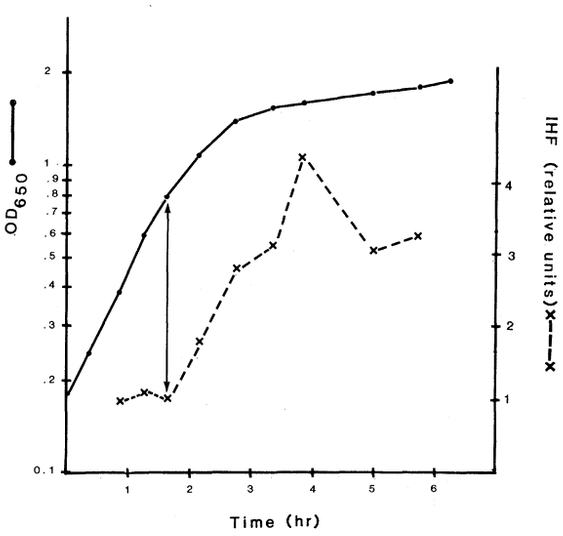

Fig. 7. Intracellular IHF as a function of growth phase. *E. coli* strain HN356 (*9*) was grown in rich medium, and optical density (OD) was measured at 650 nm. A double-headed arrow shows the last point during exponential phase growth. Cells (constant wet weight) were harvested (nonlinearity of light scattering at high cell density was taken into account by diluting cells to determine the volume needed), centrifuged (SS34 rotor) at 4000 rev/min, and resuspended in 10 m*M* tris (*p*H 7.9) containing 1 m*M* EDTA. Cells were lysed on ice by addition of NaOH to 0.1*M* and vortexed for 1 minute. The cells were put on ice for 15 minutes and HCl was added to 0.2*M*; they were then vortexed for 1 minute. After sedimenting the cellular debris in a microfuge, the supernatant was neutralized with tris base and assayed for DNA binding activity in a solution with 5×10^{-13}*M* labeled DNA containing IHF binding sites, unlabeled salmon sperm DNA (100 μg/ml), 100 m*M* NaCl, 10 m*M* $MgCl_2$, and 50 m*M* tris (*p*H 7.9). These samples were then subjected to electrophoresis on 5 percent polyacrylamide gels as described (*24*). The DNA binding patterns of the in vivo extracts were compared to samples containing known amounts of IHF. With this method, the yield of IHF from exponential phase cells is approximately the same as that obtained in the original IHF purification (*9*). The ratio between IHF present in the stationary (as compared to the exponential phase) varied between 3 and 10 in different experiments.

The observation of IHF inhibition takes on added significance when the concentrations of IHF in vivo are examined. The amount of IHF per cell is stable during exponential growth; but there is a four- to eightfold increase in the amount of IHF as cells enter the stationary phase (Fig. 7). Similar results are found when cells are grown in minimal medium containing either glucose or glycerol as the carbon source. It is not yet clear how this increase in intracellular IHF concentration is related to its various roles in *E. coli*; the magnitude of the change suggests that it may be relevant to the biological control of recombination, because the fourfold increase in IHF observed in vivo is well within the minimal range necessary to go from efficient excision to efficient inhibition in vitro.

The growth-phase dependent change in IHF concentration resembles that seen with another DNA-binding protein of small size in *Escherichia coli* (*34*). H1a is present in concentrations similar (*9, 34*) to that of IHF in exponential phase cells and also increases about four- to eightfold in stationary phase. Both H1a and IHF appear capable of regulating gene expression (*34–36*) and playing a role in DNA compaction. Two additional small, heat stable DNA-binding proteins that appear to play a role in the mechanism, and possibly the regulation, of other site-specific recombination systems are HU (*37*), which shows considerable sequence homology to IHF (*38–40*), and FIS (*41*). This consistent dependence on a cellular protein suggests that each system may have evolved to include a protein whose expression pattern reflects the physiological state of the cell. With these proteins as monitors, the cellular physiology becomes an intrinsic part of the recombination reaction.

If IHF inhibition is operational in vivo, it must provide lambda with selective

advantages. IHF is present at highest concentrations when its host enters stationary phase. At first glance, it would seem that this is precisely the time when it would be most advantageous for the prophage to induce. However, in the absence of sufficient resources to produce a successful phage burst, premature excision would be lethal to the virus. If the high level of IHF is part of a general cellular signal for limited resources or synthetic capacity, inclusion of a system responsive to its concentration would be a useful element in the viral regulatory pathway (*42–45*). The analogy has been drawn between prophage induction and abandoning a ship in distress (*46*). To extend this analogy, IHF inhibition is a mechanism for the prophage to monitor the availability of a lifeboat, that is, the presence of sufficient cellular resources for production of virus particles, before committing itself. Consistent with the suggested regulatory role of IHF inhibition is the observation that prophage induction is reduced in nutritionally deficient cells (*47*). In any in vivo experiments of this type, it is difficult to distinguish the precise role of IHF from factors influencing the amounts of Xis and Int (such as proteolysis and levels of protein synthesis). With the recent cloning of the IHF genes under inducible promoters, it should be possible to more directly test these regulatory mechanisms in vivo (*38, 39*).

Regulation as an intrinsic element in recombination mechanisms. Our data show that integrative and excisive recombination proceed along discrete pathways that differ not only in their dependence on the presence or absence of Xis, but also in their response to changes in the concentration of IHF, as well as Int (*48*). The two pathways also differ in the set of protein binding sites required for recombination. The *att*P site requires the full extent of P arm binding sites for integration while P2-B′, which lacks P1 and H1, is as good or better a substrate for excision than P1-B′ under all conditions tested. Both X1-B′ and X2-B′ are competent for excision, but under a more restricted set of conditions (Table 1). The differential use of protein binding sites had been inferred from the behavior of a resected *att*P which had *att*R-like properties in certain recombination conditions (*27*) and, more recently, has also been found in the in vivo and in vitro studies of DNA's with point mutations in different Int binding sites (*29, 30*).

As predicted from the nonequivalence of the integrative and excisive pathways, there exist combinations of *att* sites that are intrinsically unidirectional, regardless of the proteins present. For example, the *att*R deleted for P1 and H1 yields efficient excisive recombination with *att*L, but the products of this event cannot carry out integrative recombination. Similarly, we have constructed an *att*P mutated in the Xis site which can recombine only in the integrative mode (*28*).

Although the excisive recombination reaction requires neither P1 nor H1, a more complete view of prophage excision must incorporate the phenomenon of IHF inhibition. In any regulatory scheme, the inhibitory signals are no less important than the stimulatory signals. P1 and H1 are relevant for the excision reaction in that they allow for its inhibition under certain conditions. Thus, the integration and excision pathways make use of the same protein binding sites, but in vastly different ways.

The marked difference between integration and excision in their dependence on all three proteins means that alterations in the concentration of any one alone will not allow reversal of recombination. Only when concentrations of more than one protein are changed significantly will recombination in the other direction be favored. Once either reaction has been carried out, the reverse reaction cannot occur for a significant length of time; for example, until Xis and IHF levels change substantially and in opposite directions. As a consequence of the fact that the forward and reverse reactions proceed along discrete pathways, site specific recombination functions well as a long-term mode of genetic regulation that can be altered only after significant physiological changes have taken place.

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